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NATIONAL CANCER INSTITUTE
ANNUAL REPORT

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October 1, 1980 through September 30, 1981

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ANNUAL REPORT

DIVISION OF CANCER TREATMENT

October 1, 1980 through September 30, 1981

The Division of Cancer Treatment is the component of the National Cancer Institute with primary responsibility for the development and evaluation of new methods of cancer treatment. Research activities encompass all modes of therapy, including surgery, radiotherapy, chemotherapy and immunotherapy, both individually and in combination. The Division's responsibilities are carried out through a variety of mechanisms. Investigator-initiated laboratory and clinical research is performed under the auspices of traditional research grants. Targeted activities of the Division, such as drug development, are carried out through contracts and clinical Cooperative Group grants. Complementary to these extramural programs are the activities, both laboratory and clinical, carried out by the DCT intramural staff.

Personnel and Organization

The Division currently consists of five operating programs, each headed by an Associate Director. A current organization chart is shown in Figure 1. Numerous changes have occurred throughout the Division during the past year, and they will be listed here according to program area.

A. Office of the Director

1. Dr. Bruce Chabner was appointed Acting Director as of June 17, 1981. Dr. Saul Schepartz, Acting Director since January 1980, returned to his regular position as Deputy Director.

2. Ms. Susan Hubbard was appointed Chief of the recently organized Scientific Information Branch, composed of the Literature Research and Publications Sections.

B. Clinical Oncology Program

1. Dr. John Ziegler, former Associate Director, left to become Editor-in-Chief, J.N.C.I., and was replaced by Dr. Bruce Chabner on September 7, 1980. Dr. Chabner was in turn replaced by Dr. Arthur Levine on an Acting basis on June 17, 1980.

2. Dr. Charles Myers was appointed Chief, Clinical Pharmacology Branch, replacing Dr. Chabner on September 7, 1980.

3. The NCI-VA Medical Oncology Branch moved its clinical operation to the National Naval Medical Center in July 1981. Its laboratory activities will be relocated in the NNMC at a later date in the next fiscal year.

C. Biological Response Modifiers Program

1. The Program was officially established as the fifth program of

the Division, with Dr. Robert Oldham appointed Associate Director. Both laboratory and clinical efforts connected with this program have commenced at the Frederick Cancer Research Center and the Frederick Memorial Hospital in Frederick, Maryland.

2. Two Branches formerly situated in the Office of the Director, DCT, became the core of this Program. They are the Biological Development Branch (Dr. Oldham, Acting Chief) and the Biological Resources Branch (Dr. John Martin, Chief).

D. Baltimore Cancer Research Program (BCRP)

Based on recommendations of previous site-visits to this program, and in view of serious problems of budget and personnel ceilings, a decision was made to phase-out the Baltimore Cancer Research Program as an intramural program and allow the staff to organize and compete as a freestanding cancer center. The clinical and laboratory research efforts of the BCRP will be supported in the future by a limited duration of contract support from DCT and by funds obtained through a regular grant application to the Cancer Centers Program.

Most BCRP staff will be transferring to the University of Maryland rolls during Summer 1981. Every effort will be made to locate suitable assignments in Baltimore, Bethesda, or Frederick for those employees desiring to remain in the government.

E. Cancer Therapy Evaluation Program (CTE)

1. Dr. Daniel Kisner was appointed Deputy Associate Director.

2. Prior to his departure for psychiatry training, Dr. Vincent Bono, Chief of the Investigational Drug Branch, transferred to the Office of the Associate Director.

3. Dr. Daniel Hoth was appointed Chief, Investigational Drug Branch.

F. Developmental Therapeutics Program (DTP)

1. Dr. Vincent Oliverio, Associate Director, resigned from this position in November of 1980 and was transferred on detail to the Division of Extramural Activities where he will be responsible for developing mechanisms for review of both resource and research contracts in that Division.

2. Dr. John Driscoll was appointed Acting Associate Director.

3. The Mammalian Genetics and Animal Production Section was elevated to the Animal Genetics and Production Branch, and Dr. Joseph Mayo was appointed Chief.

4. Dr. Michael Lowe was appointed Acting Chief of the Toxicology Branch, effective December 29, 1980.

5. Dr. Moreshwar Nadkarni was appointed Chief of the Extramural Research and Resources Branch on November 30, 1980.

Program Highlights

The individual reports of the five program areas within DCT will be presented in detail later in this report. The following summaries describe the most important accomplishments and activities of each program during fiscal year 1981 and plans for 1982. In addition, the annual reports of the activities of the Office of the Director will be briefly summarized.

Activities Located in the Office of the Director

International Treatment Research

The DCT activities in international treatment research are coordinated in the Office of the Director by Dr. Abraham Goldin, Assistant Director for International Treatment Research. These activities include liaison offices at the Institut Jules Bordet in Brussels and the Japanese Foundation for Cancer Research in Tokyo; international agreements with the USSR, France, Japan, Hungary, Federal Republic of Germany, Italy, People's Republic of China, Egypt, Poland, and the Pan American Health Organization (PAHO); and close working relationships with investigators in the United Kingdom and other countries. These programs have led to exchanges of scientists, drugs, and clinical protocols with valuable exchanges of information and a general enhancement of preclinical and clinical research in this country and abroad.

During the past year a planning meeting was held with representatives of the Federal Republic of Germany, and specific personnel have been identified for coordination of collaborative efforts, under the overall leadership of Dr. John Driscoll. Discussions have continued on the implementation of the U.S.-China agreement and a trip by NCI staff and others is planned for early FY 1982. Active collaboration has continued in particular with the USSR, France, Italy and Japan.

A detailed description of these activities may be found in Dr. Goldin's report.

Scientific Information Branch

As mentioned earlier, the Scientific Information Branch was recently established, and Ms. Susan Hubbard was appointed Chief. It consists of the Publications Section and Literature Research Section.

During the past year particular effort was devoted to elimination of the backlog that had developed in publishing Cancer Treatment Reports during the past year, a backlog caused primarily by staff turnover and delays in printing. Through changes in procedures and provision of additional personnel support, the journal should be current by later this year. In addition, efforts are currently underway to initiate a separate publication, Cancer Treatment Symposia, to relieve pressure on the primary journal.

Developmental Therapeutics Program (DTP)

The Developmental Therapeutics Program, which is responsible for the identification and preclinical development of new antitumor agents, has an extensive extramural program for acquisition, screening, toxicologic assessment, and clinical formulation of potential new antitumor agents; in addition, the DTP has an intramural component comprised of four laboratories which investigate basic aspects of tumor growth and its control by drugs.

During the past year the drug screening activities have been placed on a more rational footing through prescreening selection on the basis of unique chemical structure and computerized analysis of possible reactive groups. Of 25,000 potential agents, 12,900 were selected for screening and 5.6% were positive in P388 leukemia trials, vs. 4.4% in 1980 when less selective methods were used. For natural products, 15% of prescreened products were active in P388 vs. a rate of 2 - 3% for random materials. Three natural products (taxol, homoharringtonine and echinomycin) have entered toxicology and are expected to enter clinical trials in the coming year.

Through the NCI screening program, 10 compounds identified as active in this fiscal year have entered toxicologic testing in the new revised and abbreviated protocol for preclinical assessment. This protocol, which has been approved by the Food and Drug Administration, requires whole animal dose-finding studies in mice and dogs, gross tissue examination of these species at toxic dose levels prior to clinical trial, and histopathologic evaluation of mouse tissues during the initial Phase I trial. The change in toxicologic evaluation is expected to expedite the transition from preclinical identification of active agents to their testing in man.

New initiatives have begun in the area of preclinical screening, and address the question of the relevance of animal leukemias as compared to murine solid tumors or human tumor xenografts in nude mice in selecting active agents. In order to carry out this evaluation, two thousand compounds will be evaluated in the complete panel of mouse tumors and xenografts, an experiment that is expected to be completed in 1983.

Summary of Drug Development Accomplishments

In summary, twenty-five thousand synthetic chemical structures were evaluated as potential new antitumor agents, 12,900 of these were selectively acquired and 5.6%, or 722, were active against leukemia P388 in vivo. A total of 5,382 new extracts of natural origin was obtained for screening in addition to 424 pure compounds. Almost 12,000 fermentation products yielded 1,734 materials which were active in one or more in vitro prescreens. From this group of natural products, 950 were tested in vivo with 15% found to be active. During the year, 160 active natural product extracts were chemically fractionated and 18 new active natural products were identified.

In screening, 22,500 materials were screened in vivo for the first time and 1,800 compounds were assigned to the tumor panel. New compounds enter the panel at a rate of 250 per year and approximately 400 complete the panel each year. As of March, 1981, 670 compounds have completed the panel testing and it is now projected that the desired 2,000 compounds could complete testing by the end of 1983.

Sixteen compounds passed DN2A and entered toxicologic testing, and 6 compounds passed DN2B and are ready to enter toxicology studies.

Intramural Activities - DTP

The mechanism of action of m-AMSA, a drug with proven activity against acute myelocytic leukemia, has been elucidated by Csyk and colleagues. The drug is activated by microsomal N-hydroxylation to a highly reactive species which is capable of covalent binding to glutathione or to nucleophilic macromolecular sites. A second drug of clinical interest, PALA, has received further scrutiny. This inhibitor of de novo pyrimidine synthesis has proven to be essentially inactive in clinical trials. When L1210 tumor cells were inoculated in medium containing $^{14}\text{C-HCO}_3$ and uridine at physiologic concentrations, the DNA pyrimidines were primarily derived from the uridine pool, indicating that a block of the de novo pathway is likely to have little effect on cells which can salvage pyrimidines from the circulating pool. Continued evaluation of drugs and potential carcinogens in primates has conclusively demonstrated the carcinogenicity of procarbazine, methylnitrosourea, and several nitrosamine derivatives. This colony of animals continues to be a unique resource for evaluation of carcinogenic potential of antitumor drugs.

In conjunction with Phase I studies of aziridiny] benzoquinone (see report of the Clinical Oncology Program), pharmacokinetic assessment was conducted. The primary elimination $t_{1/2}$ from plasma was 33.3 minutes and was dose dependent. Little unchanged AZQ was found in urine (less than 0.2% of dose). Total body clearance was 517 ml/min, suggestive of hepatic clearance or peripheral tissue metabolism as a primary elimination process.

Drug synthesis efforts have resulted in the production of new tight binding inhibitors of cytidine deaminase, an important enzyme in the inactivation of cytidine analogs, and compounds which are oxidized by tyrosinase, an enzyme present in melanosomes and in malignant melanoma cells.

Studies have continued on the mechanisms of action of DON, (6-diazo5-oxo-L-norleucine), an inhibitor of purine synthesis, and on sangivamycin, a drug of unknown mechanism. Clinical interest in the latter drug may be re-awakened by the finding that this drug is maximally active when given as a prolonged infusion, since inhibition of nucleic acid synthesis by sangivamycin depends on drug concentration and duration of exposure.

The Laboratory of Tumor Cell Biology has reported important discoveries of retrovirus isolates from human T cell lymphomas. One of these viruses was able to infect normal T cells, and antibodies to viral proteins were found in sera of patients with cutaneous T cell lymphoma-leukemia. This laboratory has also made progress in the cloning of unique DNA sequences associated with transformation of monkey cells, and in the cloning of human "onc" gene sequences analogous to the sarc genes associated with simian sarcomas.

T cell growth factor has been purified to homogeneity and its growth stimulating properties and regulation of its action further characterized. This factor, first described by the LTCB, has become a fundamental tool for expanding T lymphocytic populations in vitro.

Cancer Therapy Evaluation Program

The Cancer Therapy Evaluation Program (CTEP) is responsible for all grant- and contract-supported extramural clinical activities of the DCT. The CTEP assembles preclinical information required by the FDA and serves as a liaison with that organization. It is responsible for the initiation and monitoring of all clinical trials conducted under the auspices of the Division. These trials include Phase I and Phase II clinical trials and, in some instances, disease-specific Phase III clinical trials under contract. In addition, the grant-supported Clinical Cooperative Group Program and R01 and P01 clinical grants are in this area.

The personnel and organization changes in CTEP have been recounted earlier in this report. All personnel and offices have moved to the fourth floor of the Landow Building. Included are: Clinical Investigation Branch (CIB), Investigational Drug Branch (IDB), Radiotherapy Development Branch (RDB), and Biologics Evaluation Branch (BEB). The latter branch was created to serve as the coordinating administration structure for clinical trials with biological response modifiers. Dr. Macdonald is the Acting Branch Chief and Drs. Bruno and Poster have been transferred from IDB to staff the branch. Other changes within the CTEP include the assignment of Dr. Daniel Kiser as Deputy Associate Director, CTEP, the appointment of Dr. Daniel Hoth as Chief, IDB, and the retirement effective July 1, 1981, of Dr. Roger Halterman. The activities of the RDB have expanded significantly since Dr. David Pistenma became Chief in August, 1979. Organizational changes include the addition of Dr. Edward Gilbert, a clinical radiation therapist to the program. The IDB has undergone two organizational changes with Dr. Hoth becoming Branch Chief and Dr. Silvia Marsoni, Section Chief of the Drug Evaluation and Reporting Section. Dr. Vincent Bono was transferred to the OAD as Special Assistant for Information Management, prior to his beginning a psychiatry residency. These changes are intended to strengthen the CTEP activities in the specific areas of clinical trials monitoring, extramural radiotherapy research, and relationship to the Food and Drug Administration in the area of new drug evaluation.

A new clinical trials program, using the task order mechanism, has been set up in conjunction with the BRMP, and trials for evaluating thymosin, pyran copolymer MVE-2, and interferon have been initiated. A separate Phase I

trial program for pediatric patients has been initiated by the contract mechanism as the result of new evidence that children in general show considerably enhanced tolerance to drugs. Phase II trials of laetrile have been completed and failed to disclose evidence of clinical activity.

Tetrahydrocannabinol is now being supplied as an antiemetic to 600 hospital pharmacies under the "Group C" system, approved by the FDA in 1980. A request for surgical planning grant proposals has been issued and initial support for surgical oncology programs is expected to be allocated in the coming year.

Major changes in the funding mechanism of cooperative clinical trials have been proposed and will be initiated in 1981-1982. These include the establishment of regional clinical trials groups and a transfer of all group funding to a cooperative agreement mechanism. IND applications were filed for 5-methyl tetrahydrohomofolate and spirogermanium in the first quarter of fiscal 1981. An additional 7 IND's are expected to be filed in the final months of 1981 and the first quarter of 1982.

Two chemoprevention programs in cervical and skin cancer have been initiated.

Scientific Highlights in CTEP

1. The GI Tumor Study Group has shown, in a study of 165 patients with resected gastric cancer, that adjuvant therapy with 5-FU plus methyl CCNU increases significantly the time to recurrence.
2. In a study of patients with rectal cancer, the GI Tumor Study Group has shown that the combination of postoperative radiation with 5-FU and methyl CCNU was superior to surgery alone, with a recurrence rate of 21% vs. 52%. This controlled randomized study provides the first documented evidence of the objective benefit of postoperative therapy in rectal cancer.
3. The National Surgical Adjuvant Breast Cancer Program reported the results of its chemo-hormonal therapy adjuvant study. In patients with Stage II breast cancer a combination of phenylalanine mustard, fluorouracil, and tamoxifen was shown to be superior to phenylalanine mustard plus fluorouracil. This benefit in disease-free survival corresponded directly with increasing estrogen receptor protein level measured in the resected tumor tissue. This study has defined a role for an antiestrogen therapy in the adjuvant treatment of breast cancer.
4. A Brain Tumor Study Group trial in patients with malignant gliomas following maximal surgical resection suggest that BCNU plus radiotherapy is better than radiotherapy alone.
5. Another Brain Tumor Study Group trial in patients with gliomas showed that, although corticosteroids do not improve survival, they can be used effectively and safely to produce the known acute benefits.

Clinical Oncology Program (COP)

The Clinical Oncology Program of the National Cancer Institute conducts clinical and laboratory investigations into the etiology, diagnosis, and treatment of human malignancies. The program consists of seven branches, which carry out laboratory and clinical studies either independently or in collaboration with the other branches of the program.

The primary organization change in the past year has been the assignment of a senior statistician from the Biometrics Research Branch to each of the clinical branches to aid in the design and analysis of all clinical trials.

Important laboratory advances include:

1. The identification of polyglutamate metabolites of methotrexate. These metabolites are selectively retained by tumor cells in the absence of free extracellular drug and may influence the duration of action and cytotoxicity of the drug.
2. A new Se-independent glutathione peroxidase has been isolated and purified from mitochondrial membrane and forms a major detoxifying mechanism for free radicals.
3. Comprehensive human pharmacokinetic studies of misonidazole, adriamycin, 5-FU, and 13-Cis-retinoic acid in support of ongoing clinical trials.
4. Characterization of a unique chromosomal abnormality in human small cell carcinoma (deletion of 3p).
5. Isolation of hormone-resistant breast cancer cell lines with defective receptor proteins.
6. Further characterization of the pivotal role of antigen-presenting cells in the response of T cells.
7. Establishment of methods for reliable in vitro growth and cloning of human small cell carcinoma cell lines, and karyotypic and biochemical characterization of these lines.
8. Development of methods for cloning and expanding single lymphoid cells in culture using T cell growth factor; cytotoxic T cells can also be expanded.
9. Demonstration that the positioning of patients after lumbar puncture has significant impact on the equilibration of drug with intracerebral spinal fluid.
10. Isolation of transformed hybrid cell lines of hamster cells which display various degrees of oncogenicity as a function of the expression of specific viral antigens.

The major clinical findings of the past year included the following:

1. Major extensions of disease-free survival through the use of combination chemotherapy, surgery and radiation in the adjuvant therapy of soft tissue sarcoma.
2. Improved complete remission rates in histiocytic lymphoma, Ewing's sarcoma, ovarian carcinoma, and childhood lymphomas using intensive combination chemotherapy.
3. Promising improvement in childhood ALL duration of complete remission, and effective prevention of CNS leukemia, using a regimen which includes high-dose systemic methotrexate.
4. Phase I trials of AZQ, pentamethylmelamine, ICRF-187, misonidazole, and intraperitoneal 5-FU have been completed.

Biological Response Modifiers Program - (BRMP)

The BRMP, as recommended by the Board of Scientific Counselors of DCT and its Subcommittee on Biological Response Modifiers, has become fully operational in fiscal year 1981 with the appointment of Dr. Robert Oldham as Associate Director for this program, the appointment of 14 senior scientists, occupancy of 6000 sq. feet of laboratory space at the Frederick Cancer Research Center, and the initiation of clinical research at the Frederick Memorial Hospital. In addition, extramural activities associated with BRM's, some of which were formerly located in other divisions of the NCI, have been centralized in the grant and contract activities of the DCT, and now total approximately \$10.8M in awards for this fiscal year.

In conjunction with the intention to assess BRM's as potential antitumor agents, an organizational framework has been established to provide for acquisition, screening, and preclinical toxicologic assessment of new agents. In addition, the BRMP has initiated trials of highly purified lymphoblastoid interferon supplied by Hoffman-LaRoche at NCI units in Frederick, Baltimore and Bethesda. Evaluation of less purified preparations of human leukocyte interferon and lymphoblastoid interferon were initiated during the current fiscal year under sponsorship of the BRMP through the Cancer Therapy Evaluation Program. In studies using material supplied by the American Cancer Society, responses have been noted in nodular lymphomas, multiple myeloma, and breast cancer, although the significance and long-term benefits of interferon as an antitumor agents are impossible to assess at this time. The NCI studies are designed to provide this assessment.

In addition to the organization of its program and initiation of intramural research activities, the BRMP sponsored a series of six workshops on various aspects of immunologic approaches to cancer treatment and the role of biologic growth and maturation factors in regulating tumors.

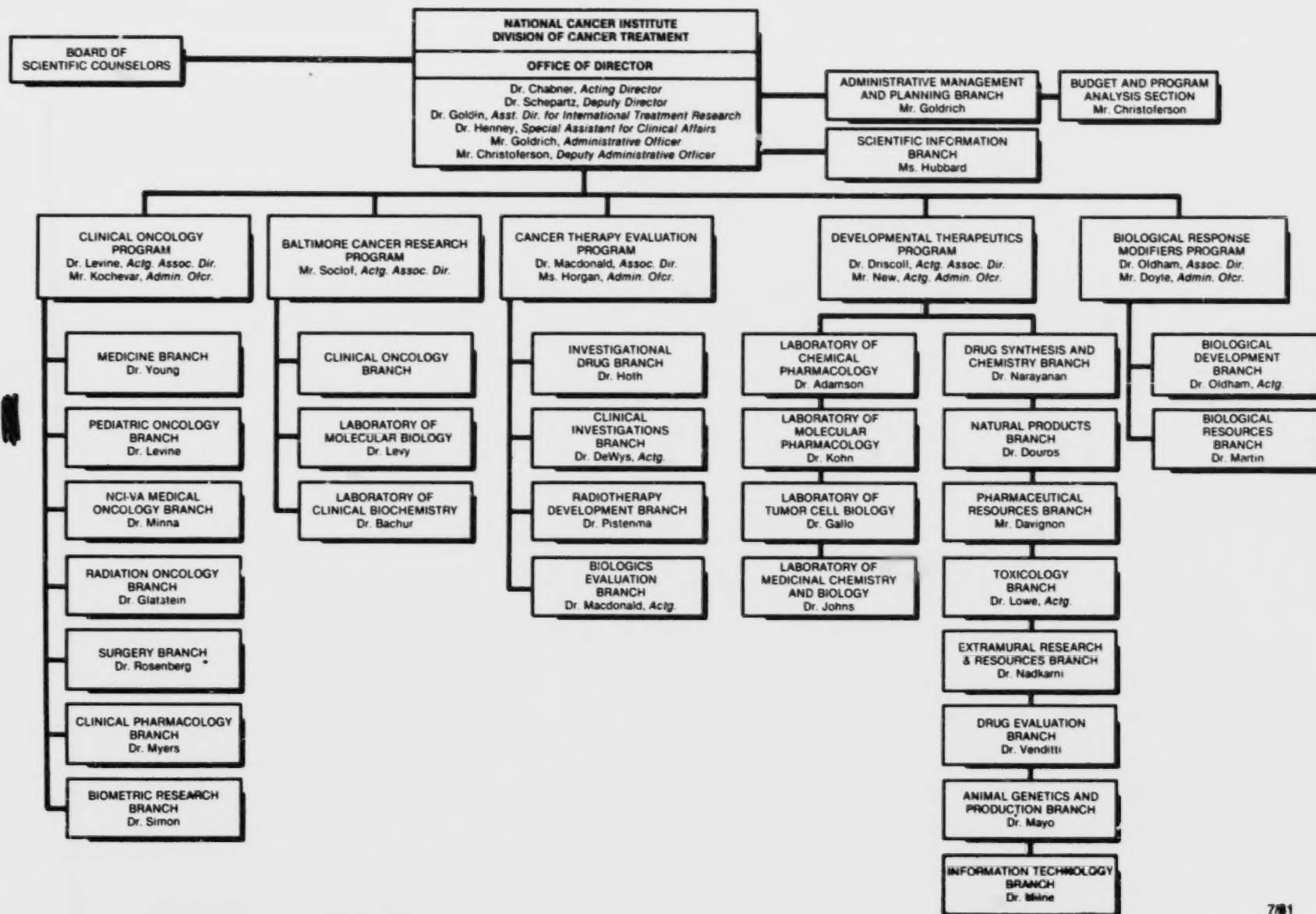
Baltimore Cancer Research Program (BCRP)

The BCRP will become a free standing cancer center in July of 1981, with phase out funding from the DCT to continue over the next 3 years, as explained earlier in this report. The Program continues to make notable contributions to the treatment of acute leukemia and lymphoma, and the development of new therapies through Phase I-II trials.

1. New drugs, including AMSA, dihydroxyanthracenedione, aclacinomycin, vindesine and ADC have undergone Phase I-II testing.
2. Current studies in adult acute leukemia are evaluating various maintenance schemes, including late intensification therapy, splenectomy, and immunotherapy with neuraminidase-treated leukemic cells.
3. Evaluation of a recently completed Hodgkin's disease study comparing chemotherapy with radiotherapy plus chemotherapy shows that MOPP chemotherapy and radiotherapy are equally effective in producing complete responses in patients with stage II-IIIA Hodgkin's disease.
4. Active new drug combinations have been identified for metastatic breast, ovarian, cervical and bladder cancer.
5. Autologous frozen platelets in acute leukemia patients without compatible donors are effective in the prevention of bleeding.

Productive laboratory investigations continue on the mechanism of action of anthracyclines, their activation by enzymatic mechanisms, and their interaction with metals.

Figure 1



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Division of Cancer Treatment Stratification

<u>Code</u>	<u>Description</u>
PDD	Preclinical Drugs Development Program -
1XX	Stage I - Acquisition of Materials
2A1	Stage II - Basic Screen - Determination of anti-tumor activity of new agents.
2B1	- Develop acceptable experimental formulation.
2B2	- Verification screen - Detailed evaluation of new agents (dose, route and schedule dependency).
2B3	- Procurement of sufficient amounts of new agent for preclinical studies.
3X1	Stage III - Toxicology and pharmacology in animals.
3X2	- Production and formulation for clinical trials.
PBR	Preclinical Basic Research -
PCS	Cellular/Subcellular Studies - Includes biochemistry, biological response modifiers, blood products, cell biology, cell kinetics, therapeutic nutrition, immunobiology, markers, molecular biology, radiobiology, transfusion research, hyperthermia, combined modalities, radiation modifiers, radiation immunology, radiation physics, comparative pharmacology, experimental therapy, mechanism of drug action, synthetic and natural products, and data processing.
PTS	Treatment Studies - Independent treatments, combined modality therapy, radiation physics, radiation equipment development, nuclear medicine and data processing.
CTR	Clinical Trials Research -
CT1	Phase I Clinical Trials* - Initial clinical evaluation of new drugs, clinical pharmacology.
CT2	Phase II Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy (single agents or combinations) or combined modality regimens having highest priority for initial efficacy evaluation.
CT3	Phase III Clinical Trials* - Allocation for specific disease-oriented resources to study whatever chemotherapy or combined

or combined modality regimens have highest priority for efficacy evaluation in a controlled clinical setting.

- CT4 Phase IV Clinical Trials* - Allocation for specific disease-oriented resources to evaluate the combined modality approach to the initial therapeutic attack on local or regional disease in an attempt to increase the number of patients with a long disease-free period.
- CT5 Statistics, Data Processing, and Other Clinical Trials Research
- CSR Clinical Trials Supportive Research - Includes special pharmacology/toxicology, cell kinetics, markers, blood products, transfusion research, protected environment, hyperthermia, nutrition, statistics, and data processing.
- MGT Program Management - Includes administration, dissemination of information to the medical and scientific community.

*Supportive care used as ancillary therapy should be prorated among the phases of clinical trials using such resources.

TABLE I
ANALYSIS OF CONTRACT ACTIVITIES FOR FY81

	ANNUAL LEVEL *	PERCENT
PDD PRECLINICAL DRUG DEVELOPMENT PROGRAM	38,763,500	72.61
STAGE I	11,082,302	20.65
1XX ACQUISITION OF MATERIALS		
STAGE IIA	10,679,159	19.90
2A1 DETERMINATION OF ANTI-TUMOR ACTIVITY		
STAGE IIB	8,611,084	16.05
2B1 EXPERIMENTAL FORMULATION DEVELOPMENT	362,111	0.67
2B2 DETAILED EVALUATION OF NEW AGENTS	5,071,786	9.45
2B3 PROCURE. OF AGENTS FOR PRECLIN. STUDIES	3,177,187	5.92
STAGE III	8,590,955	16.01
3X1 TOXICOLOGY & PHARM. IN LARGE ANIMALS	4,623,475	8.62
3X2 PROD. & FORM. FOR CLINICAL TRIALS	3,967,480	7.39
PBR PRECLINICAL BASIC RESEARCH	3,082,631	5.74
PBS CELLULAR/SUBCELLULAR STUDIES	1,415,508	2.64
PTS TREATMENT STUDIES	1,667,123	3.11
CTR CLINICAL TRIALS RESEARCH	9,928,825	18.50
CT1 PHASE I CLINICAL TRIALS	1,291,279	2.41
CT2 PHASE II CLINICAL TRIALS	1,729,555	3.22
CT3 PHASE III CLINICAL TRIALS	3,229,134	6.02
CT4 PHASE IV CLINICAL TRIALS	2,569,680	4.79
CT5 OTHER CLINICAL TRIALS RESEARCH	1,109,177	2.07
CSR CLINICAL TRIALS SUPPORTIVE RESEARCH	977,110	1.82
MGT PROGRAM MANAGEMENT	711,935	1.33
TOTAL	53,664,001	100.00

* FY-81 FUNDING REQUIREMENTS AS OF MAY 31, 1981 FOR ABOUT 220 CONTRACTS. NOT INCLUDED ARE ABOUT 17 SCHEDULE A CONTRACTS (\$5,865,604) AND 16 SCHEDULE B CONTRACTS (\$4,222,031). ALSO NOT INCLUDED ARE FUNDS UTILIZED FOR DIRECT PURCHASE OF CLINICAL DRUGS (ABOUT \$2,050,000) AND ONE UNCLASSIFIED CLINICAL ONCOLOGY PROGRAM CONTRACT FOR \$969,000.

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TABLE II
ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:		
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001	406,039
D.T.P.	ALABAMA, UNIVERSITY OF	N01CM07355	17,461
A.P.	ALABAMA, UNIVERSITY OF	N01CP95616	3,000
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM97262	128,000
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM97297	143,381
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM07346	78,840
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	10,399
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM07266	141,124
D.T.P.	BRISTOL LABORATORIES	N01CM07299	446,049
D.T.P.	BRISTOL LABORATORIES	N01CM07324	221,000
A.P.	CHARLES RIVER BREEDING LABS.	N01CM17498	30,108
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	49,842
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	103,939
A.P.	CHARLES RIVER BREEDING LABS.	N01CM90163	47,502
A.P.	CHARLES RIVER BREEDING LABS.	N01CM97229	73,928
D.T.P.	CHEMICAL ABSTRACTS SERVICE	N01CM43722	522,000
D.T.P.	COLLABORATIVE RESEARCH, INC.	N01CM07358	14,984
D.T.P.	ENVIRO CONTROL, INC.	N01CM07332	94,887
D.T.P.	FLOW LABORATORIES, INC.	N01CM97254	225,664
A.P.	HARLAN INDUSTRIES	N01CM07362	111,483
A.P.	HARLAN INDUSTRIES	N01CM50591	43,992
A.P.	HARLAN INDUSTRIES	N01CM97242	55,166
A.P.	HARLAN INDUSTRIES	N01CM97243	46,116
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	95,934

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
STAGE I AQUISITION OF MATERIALS:			
A.P.	HEALTH RESEARCH, INC.	N01CM77101	757
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97213	59,890
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97316	146,007
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM97259	99,837
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM97295	131,379
O.D.	INSTITUT JULES BORDET	N01CM53840	13,440
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM43736	52,500
CTEP	INSTITUTE OF CANCER RESEARCH	N01CM77139	17,000
D.T.P.	IOWA, UNIVERSITY OF	N01CM07412	121,000
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	1,600
A.P.	KING ANIMAL LABORATORY	N01CM17499	28,704
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	44,928
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	41,795
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	160,726
D.T.P.	LITTON BIONETICS, INC.	N01CM07326	149,400
D.T.P.	LITTON BIONETICS, INC.	N01C075380	1,699,200
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N01CM97317	68,280
BRMP	MELOY LABORATORIES, INC.	N01CM07378	56,000
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	19,264
D.T.P.	MICHIGAN TECHNOLOGICAL UNIVERSITY	N01CM07293	95,000
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	47,050
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97287	55,125
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	22,389
A.P.	MISSOURI, UNIVERSITY OF	N01CM97211	18,053

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	STAGE I AQUISITION OF MATERIALS:			
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	44,142	
A.P.	NORTHROP SERVICES, INC.	N01CM07286	13,120	
A.P.	NORTHWESTERN UNIVERSITY	N01CM17363	9,579	
D.T.P.	POLYSCIENCES, INC.	N01CM07300	270,096	
D.T.P.	PURDUE RESEARCH FOUNDATION	N01CM97296	156,386	
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM07352	6,705	
A.P.	SASCO, INC.	N01CM90164	46,800	
A.P.	SIMONSEN LABORATORIES	N01CM50578	49,140	
A.P.	SIMONSEN LABORATORIES	N01CM77166	162,060	
A.P.	SIMONSEN LABORATORIES	N01CM97247	71,618	
D.T.P.	SISA, INC.	N01CM07354	16,074	
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	100,800	
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	46,332	
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	32,928	
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM07260	25,100	
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	20,082	
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM07351	14,919	
CTEP	STANFORD RESEARCH INSTITUTE	N01CM87207	60,160	
D.T.P.	STARKS ASSOCIATES, INC.	N01CM07357	24,334	
D.T.P.	STARKS ASSOCIATES, INC.	N01CM87206	555,000	
A.P.	TACONIC FARMS	N01CM50597	5,100	
D.T.P.	UPJOHN COMPANY	N01CM07380	682,724	
D.T.P.	VSE, CORPORATION	N01CM07251	144,524	
BRMP	WARNER LAMPERT	N01CM07292	37,190	

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81			DOLLAR LEVEL
AREA	CONTRACT				
STAGE I AQUISITION OF MATERIALS:					
D.T.P.	WARNER LAMPERT		N01CM07379		583,950
BRMP	WELLCOME FOUNDATION, LTD.		N01CM17489		1,649,277
TOTAL					11,082,302

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	#	DOLLAR LEVEL
	STAGE II BASIC SCREEN:				
A.P.	ALABAMA, UNIVERSITY OF			N01CP95616	8,250
D.T.P.	ARIZONA STATE UNIVERSITY			N01CM17497	245,565
CTEP	ARTHUR D. LITTLE, INC.			N01CM07257	58,217
D.T.P.	ARTHUR D. LITTLE, INC.			N01CM07302	268,101
D.T.P.	ARTHUR D. LITTLE, INC.			N01CM07331	105,834
D.T.P.	ARTHUR D. LITTLE, INC.			N01CM07346	289,082
D.T.P.	BATTELLE MEMORIAL INSTITUTE			N01CM07266	517,454
D.T.P.	BATTELLE MEMORIAL INSTITUTE			N01CM67099	520,930
D.T.P.	BRISTOL LABORATORIES			N01CM07299	78,714
D.T.P.	CALIFORNIA, UNIVERSITY OF			N01CM07420	217,350
D.T.P.	CANCER THERAPY & RESEARCH FOUND. OF SOUTH TEXAS			N01CM07327	312,563
A.P.	CHARLES RIVER BREEDING LABS.			N01CM17498	82,797
A.P.	CHARLES RIVER BREEDING LABS.			N01CM50598	137,066
A.P.	CHARLES RIVER BREEDING LABS.			N01CM77141	285,831
A.P.	CHARLES RIVER BREEDING LABS.			N01CM90163	130,631
A.P.	CHARLES RIVER BREEDING LABS.			N01CM97229	203,302
CTEP	FOX CHASE CANCER CENTER			N01CM07330	72,215
A.P.	HARLAN INDUSTRIES			N01CM07362	306,577
A.P.	HARLAN INDUSTRIES			N01CM50591	120,978
A.P.	HARLAN INDUSTRIES			N01CM97242	151,707
A.P.	HARLAN INDUSTRIES			N01CM97243	110,319
A.P.	HEALTH RESEARCH, INC.			N01CM77101	2,081
D.T.P.	IIT RESEARCH INSTITUTE			N01CM97213	194,643
D.T.P.	IIT RESEARCH INSTITUTE			N01CM97316	691,098

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
STAGE II BASIC SCREEN:				
D.T.P.	INSTITUT JULES BORDET	N01CM07350	159,429	
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	6,400	
A.P.	KING ANIMAL LABORATORY	N01CM17499	78,936	
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	123,552	
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	114,936	
A.P.	LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165	441,996	
D.T.P.	LITTON BIONETICS, INC.	N01CM075380	424,800	
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	15,890	
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N01CM97317	833,011	
D.T.P.	MAYO FOUNDATION	N01CM07419	184,544	
D.T.P.	MICHIGAN TECHNOLOGICAL UNIVERSITY	N01CM07293	5,000	
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	129,388	
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97287	151,594	
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	61,570	
A.P.	MISSOURI, UNIVERSITY OF	N01CM97211	49,646	
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	121,390	
A.P.	NORTHROP SERVICES, INC.	N01CM07286	36,081	
A.P.	NORTHWESTERN UNIVERSITY	N01CM17363	26,343	
A.P.	SASCO, INC.	N01CM90164	128,700	
A.P.	SIMONSEN LABORATORIES	N01CM50578	135,135	
A.P.	SIMONSEN LABORATORIES	N01CM77166	445,664	
A.P.	SIMONSEN LABORATORIES	N01CM97247	196,950	
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	127,413	
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	90,552	

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
STAGE II BASIC SCREEN:			
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	642,636
A.P.	TACONIC FARMS	N01CM50597	14,025
D.T.P.	UPJOHN COMPANY	N01CM07380	120,481
D.T.P.	VSE, CORPORATION	N01CM07251	598,742
D.T.P.	WARNER LAMPERT	N01CM07379	103,050
TOTAL			10,679,159

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ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
STAGE II FORMULATION:			
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM97298	85,677
D.T.P.	IOWA, UNIVERSITY OF	N01CM07303	63,358
D.T.P.	KANSAS, UNIVERSITY OF	N01CM07304	75,235
D.T.P.	KENTUCKY, UNIVERSITY OF	N01CM07381	75,945
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	19,896
D.T.P.	YAMANOUCI PHARMACEUTICAL CO.	N01CM97307	42,000
TOTAL			362,111

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:			
A.P.	ALABAMA, UNIVERSITY OF	N01CP95616	3,750	
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM17497	27,285	
CTEP	ARTHUR D. LITTLE, INC.	N01CM07257	135,841	
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM07331	6,755	
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM07346	131,401	
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM07266	235,206	
D.T.P.	CALIFORNIA, UNIVERSITY OF	N01CM07420	24,150	
D.J.P.	CANCER THERAPY & RESEARCH FOUND. OF SOUTH TEXAS	N01CM07327	34,729	
A.P.	CHARLES RIVER BREEDING LABS.	N01CM17498	37,635	
A.P.	CHARLES RIVER BREEDING LABS.	N01CM50598	62,303	
A.P.	CHARLES RIVER BREEDING LABS.	N01CM77141	129,923	
A.P.	CHARLES RIVER BREEDING LABS.	N01CM90163	59,378	
A.P.	CHARLES RIVER BREEDING LABS.	N01CM97229	92,410	
CTEP	FOX CHASE CANCER CENTER	N01CM07330	18,054	
A.P.	HARLAN INDUSTRIES	N01CM07362	139,353	
A.P.	HARLAN INDUSTRIES	N01CM50591	54,990	
A.P.	HARLAN INDUSTRIES	N01CM97242	68,958	
A.P.	HARLAN INDUSTRIES	N01CM97243	50,145	
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	47,967	
A.P.	HEALTH RESEARCH, INC.	N01CM77101	946	
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97213	44,918	
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97316	107,072	
D.T.P.	INSTITUT JULES BORDET	N01CM07350	39,857	
D.T.P.	INSTITUTE OF CANCER RESEARCH	N01CM43736	52,500	

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	STAGE II VERIFICATION SCREEN:		
A.P.	KING ANIMAL LABORATORY	N01CM17499	35,880
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM50577	56,160
A.P.	LABORATORY SUPPLY COMPANY, INC.	N01CM97244	52,244
A.P.	LEO GODWIN INST. FOR CANCER RESEARCH	N01CM77165	200,907
D.T.P.	LITTON BIONETICS, INC.	N01C075380	1,062,000
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N01CM07325	202,922
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N01CM97317	327,742
D.T.P.	MAYO FOUNDATION	N01CM07419	20,505
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97246	58,813
A.P.	MICROBIOLOGICAL ASSOCIATES	N01CM97287	68,906
A.P.	MISSOURI, UNIVERSITY OF	N01CM87157	27,986
A.P.	MISSOURI, UNIVERSITY OF	N01CM97211	22,566
A.P.	MURPHY BREEDING LABS., INC.	N01CM50579	55,177
A.P.	NORTHROP SERVICES, INC.	N01CM07286	16,400
A.P.	NORTHWESTERN UNIVERSITY	N01CM17363	11,974
A.P.	SASCO, INC.	N01CM90164	58,500
A.P.	SIMONSEN LABORATORIES	N01CM50578	61,425
A.P.	SIMONSEN LABORATORIES	N01CM77166	202,575
A.P.	SIMONSEN LABORATORIES	N01CM97247	89,523
A.P.	SOUTHERN ANIMAL FARMS	N01CM50599	57,915
A.P.	SOUTHERN ANIMAL FARMS	N01CM97245	41,160
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	522,142
A.P.	TACONIC FARMS	N01CM50597	6,375
D.T.P.	VSE, CORPORATION	N01CM07251	206,463

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	CONTRACT	ACTIVITY FOR FISCAL YEAR 1981 AS OF 05/31/81	#	DOLLAR LEVEL
		TOTAL		5,071,786

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	#	DOLLAR LEVEL
	STAGE II PROCUREMENT OF PRECLINICAL MATERIAL:				
D.T.P.	AGRICULTURE, DEPARTMENT OF	Y01CM40001		45,115	
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM17492		152,818	
D.T.P.	AMERICAN TYPE CULTURE COLLECTION	N01CM05725		80,720	
D.T.P.	ARIZONA STATE UNIVERSITY	N01CM97297		25,302	
D.T.P.	ASH STEVENS, INC.	N01CM17488		218,724	
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM17490		198,548	
D.T.P.	FLOW LABORATORIES, INC.	N01CM97254		112,832	
D.T.P.	ILLINOIS, UNIVERSITY OF	N01CM97295		23,185	
BRMP	IOWA, UNIVERSITY OF	N01CM07334		73,671	
D.T.P.	LITTON BIONETICS, INC.	N01CM05724		425,000	
D.T.P.	LITTON BIONETICS, INC.	N01CM07347		249,000	
D.T.P.	LITTON BIONETICS, INC.	N01C075380		354,000	
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM87234		50,086	
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM97255		65,534	
D.T.P.	PHARM-ECO	N01CM17487		143,733	
D.T.P.	PURDUE RESEARCH FOUNDATION	N01CM97296		27,597	
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM97313		170,056	
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM87183		110,509	
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM97256		198,056	
D.T.P.	STARKS ASSOCIATES, INC.	N01CM17374		287,895	
D.T.P.	WARNER LAMPERT	N01CM17491		164,806	
TOTAL				3,177,187	

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT		DOLLAR LEVEL
STAGE III PHARMACOLOGY/TOXICOLOGY:			
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM87163	459,242
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM17365	3,281,708
A.P.	HAZLETON LABORATORIES, INC.	N01CM60125	35,000
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	191,868
A.P.	MARSHALL RESEARCH ANIMALS, INC.	N01CM60123	25,250
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	152,940
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	7,600
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM17375	26,102
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM97264	8,400
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM87162	187,950
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97263	164,500
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	40,165
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87185	42,750
TOTAL			4,623,475

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	STAGE III PROD. AND FORM. FOR CLINICAL TRIALS:		
D.T.P.	ALDRICH CHEMICAL COMPANY, INC.	N01CM17492	152,818
D.T.P.	ASH STEVENS, INC.	N01CM17488	218,724
D.T.P.	BEN VENUE LABORATORIES, INC.	N01CM97298	771,089
D.T.P.	CORDOVA CHEMICAL COMPANY	N01CM17490	198,548
D.T.P.	FLOW LABORATORIES, INC.	N01CM67088	115,452
D.T.P.	FLOW LABORATORIES, INC.	N01CM97254	37,611
D.T.P.	IOWA, UNIVERSITY OF	N01CM07303	253,434
BRMP	IOWA, UNIVERSITY OF	N01CM07334	31,573
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	20,000
D.T.P.	MIDWEST RESEARCH INSTITUTE	N01CM87234	200,344
D.T.P.	MONSANTO RESEARCH CORPORATION	N01CM97255	589,806
D.T.P.	PHARM-ECO	N01CM17487	143,733
D.T.P.	PHILIPS ROXANE LABORATORIES, INC.	N01CM67053	79,584
D.T.P.	RESEARCH TRIANGLE INSTITUTE	N01CM97313	42,514
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM87183	442,035
D.T.P.	STANFORD RESEARCH INSTITUTE	N01CM97256	49,514
D.T.P.	STARKS ASSOCIATES, INC.	N01CM17374	287,895
D.T.P.	WARNER LAMPERT	N01CM17491	164,806
D.T.P.	YAMANOUCHI PHARMACEUTICAL CO.	N01CM97307	168,000
	TOTAL		3,967,480

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
PRECLIN. BASIC RES. - CELLULAR/SUBCELLULAR STUDIES:			
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	58,611
C.O.P.	HAZELTON LABORATORIES, INC.	N01CM15770	110,000
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	101,500
D.T.P.	HAZLETON LABORATORIES, INC.	N01CM97217	143,901
O.D.	INSTITUT JULES BORDET	N01CM53840	8,960
D.T.P.	LITTON BIONETICS, INC.	N01CM07347	249,000
C.O.P.	LITTON BIONETICS, INC.	N01CM67067	180,000
D.T.P.	LITTON BIONETICS, INC.	N01CM87187	261,880
A.P.	MASON RESEARCH INSTITUTE	N01CM87164	29,794
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	108,580
C.O.P.	MICROBIOLOGICAL ASSOCIATES	N01CM07369	100,000
D.T.P.	SMALL BUSINESS ADMINISTRATION	N01CM43719	43,200
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	20,082
TOTAL			1,415,508

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	PRECLIN. BASIC RES. - TREATMENT STUDIES:			
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM07302	219,355	
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM07346	26,280	
D.T.P.	BATTELLE MEMORIAL INSTITUTE	N01CM07266	47,041	
C.O.P.	HAZLETON LABORATORIES, INC.	N01CM87156	43,500	
D.T.P.	IIT RESEARCH INSTITUTE	N01CM97316	29,201	
C.O.P.	MASON RESEARCH INSTITUTE	N01CM67011	248,972	
D.T.P.	MASON RESEARCH INSTITUTE/EG&G	N01CM97317	136,559	
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97263	70,500	
D.T.P.	SOUTHERN RESEARCH INSTITUTE	N01CM97309	763,130	
D.T.P.	VSE, CORPORATION	N01CM07251	82,585	
TOTAL			1,667,123	

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ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81		DOLLAR LEVEL
AREA	CONTRACT		#	
PROGRAM MANAGEMENT:				
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288		14,180
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST	N01CM67054		1,206
O.D.	CDP ASSOCIATES	N01CM97143		311,867
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056		1,000
CTEP	DUKE UNIVERSITY	N01CM17477		4,308
CTEP	DUKE UNIVERSITY	N01CM67010		1,500
CTEP	INDIANA UNIVERSITY FOUNDATION	N01CM17475		5,860
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N01CM17349		5,725
CTEP	INFORMATION PLANNING ASSOC.	N01CM77104		208,979
O.D.	INSTITUT JULES BORDET	N01CM53840		22,400
CTEP	IOWA, UNIVERSITY OF	N01CM17476		9,048
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054		3,200
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058		1,200
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM17348		12,240
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290		26,269
CTEP	MONTEFIORE HOSPITAL	N01CM17474		10,432
A.P.	NATIONAL ACADEMY OF SCIENCES	N01CM53850		27,000
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y01CM00103		1,362
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM17473		9,470
CTEP	NORTH CAROLINA, UNIVERSITY OF	N01CM17471		16,158
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060		1,507
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020		2,474
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N01CM17472		14,550
TOTAL				711,935

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
PHASE I CLINICAL TRIALS:			
CTEP	ALBERT EINSTEIN SCHOOL OF MEDICINE	N01CM17340	34,333
CTEP	ARIZONA, UNIVERSITY OF	N01CM17500	23,242
D.T.P.	ARTHUR D. LITTLE, INC.	N01CM97288	11,344
CTEP	GEORGETOWN UNIVERSITY	N01CM17501	19,795
CTEP	GEORGETOWN UNIVERSITY	N01CM97208	123,191
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054	2,400
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM97272	69,064
CTEP	MAYO FOUNDATION	N01CM97273	181,805
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM97274	124,127
D.T.P.	MIAMI, UNIVERSITY OF	N01CM97290	21,015
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97275	93,141
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	60,800
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM97276	71,000
D.T.P.	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM87185	28,500
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM97277	179,630
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM97278	86,844
CTEP	WAYNE STATE UNIVERSITY	N01CM97279	90,622
CTEP	WISCONSIN, UNIVERSITY OF	N01CM97280	70,426
TOTAL			1,291,279

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	#	DOLLAR LEVEL
	PHASE II CLINICAL TRIALS:				
CTEP	ALBERT EINSTEIN SCHOOL OF MEDICINE			N01CM17340	34,333
CTEP	ARIZONA, UNIVERSITY OF			N01CM17500	23,242
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST			N01CM67054	3,617
CTEP	CALIFORNIA, UNIVERSITY OF			N01CM07416	15,097
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA			N01CM67056	3,000
CTEP	DUKE UNIVERSITY			N01CM17477	12,925
CTEP	DUKE UNIVERSITY			N01CM67010	4,500
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER			N01CM07336	30,678
CTEP	GEORGETOWN UNIVERSITY			N01CM17501	19,795
CTEP	GEORGETOWN UNIVERSITY			N01CM97208	50,317
CTEP	ILLINOIS CANCER COUNCIL			N01CM07415	22,898
CTEP	INDIANA UNIVERSITY FOUNDATION			N01CM17475	17,579
CTEP	IOWA, UNIVERSITY OF			N01CM17476	27,143
CTEP	ISTITUTO NAZIONALE TUMORI			N01CM07338	21,825
CTEP	ISTITUTO NAZIONALE TUMORI			N01CM43726	60,240
O.D.	JAPANESE FOUNDATION FOR CANCER RESEARCH			N01CM22054	2,400
CTEP	KANSAS, UNIVERSITY OF-MEDICAL CENTER			N01CM97272	28,209
CTEP	KENTUCKY, UNIVERSITY OF			N01CM67058	3,600
CTEP	MAYO FOUNDATION			N01CM07414	26,160
CTEP	MAYO FOUNDATION			N01CM43796	18,414
CTEP	MAYO FOUNDATION			N01CM57044	68,750
CTEP	MAYO FOUNDATION			N01CM97273	74,259
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES			N01CM07337	134,925
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES			N01CM17348	36,720

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
PHASE II CLINICAL TRIALS:			
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM97274	50,700
CTEP	MICHIGAN, UNIVERSITY OF	N01CM07405	126,712
CTEP	MONTEFIORE HOSPITAL	N01CM17474	31,297
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97275	38,043
D.T.P.	MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294	7,600
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y01CM00103	54,800
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM17473	28,410
CTEP	NORTH CAROLINA, UNIVERSITY OF	N01CM17471	48,474
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	4,521
CTEP	ONTARIO CANCER INSTITUTE	N01CM07418	47,821
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020	7,421
CTEP	SIDNEY FARBER CANCER INSTITUTE	N01CM97276	29,000
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N01CM17472	43,649
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM07406	152,739
CTEP	TEXAS, UNIVERSITY OF	N01CM07417	19,594
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM97277	73,370
CTEP	VERMONT, UNIVERSITY OF, COLLEGE OF MEDICINE	N01CM97278	35,472
CTEP	WAYNE STATE UNIVERSITY	N01CM07404	123,526
CTEP	WAYNE STATE UNIVERSITY	N01CM97279	37,014
CTEP	WISCONSIN, UNIVERSITY OF	N01CM97280	28,766
TOTAL			1,729,555

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:		
CTEP	ALBERT EINSTEIN SCHOOL OF MEDICINE	N01CM17340	103,000
CTEP	ARIZONA, UNIVERSITY OF	N01CM17500	69,725
CTEP	BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE FOREST	N01CM67054	7,235
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM07416	45,290
CTEP	CLINICA NEUROCHIRURGICA DELL UNIVERSITA	N01CM67056	6,000
CTEP	DUKE UNIVERSITY	N01CM17477	25,849
CTEP	DUKE UNIVERSITY	N01CM67010	9,000
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM07336	92,035
CTEP	GEORGETOWN UNIVERSITY	N01CM17501	59,384
CTEP	ILLINOIS CANCER COUNCIL	N01CM07415	68,693
CTEP	INDIANA UNIVERSITY FOUNDATION	N01CM17475	35,158
CTEP	IOWA, UNIVERSITY OF	N01CM17476	54,286
CTEP	ISTITUTO NAZIONALE TUMORI	N01CM07338	50,925
CTEP	KENTUCKY, UNIVERSITY OF	N01CM67058	7,200
CTEP	MAYO FOUNDATION	N01CM07414	78,480
CTEP	MAYO FOUNDATION	N01CM43783	17,424
CTEP	MAYO FOUNDATION	N01CM57044	56,250
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM07337	110,393
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM17348	73,440
CTEP	MICHIGAN, UNIVERSITY OF	N01CM07405	103,674
CTEP	MONTEFIORE HOSPITAL	N01CM17474	62,593
CTEP	NEW YORK UNIVERSITY MEDICAL CENTER	N01CM17473	56,820
CTEP	NORTH CAROLINA, UNIVERSITY OF	N01CM17471	96,949
CTEP	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM67060	9,042

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY	CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	PHASE III CLINICAL TRIALS:			
CTEP	ONTARIO CANCER INSTITUTE	N01CM07418		143,462
CTEP	PITTSBURGH, UNIVERSITY OF	N01CB23876		1,199,870
CTEP	SAINT LOUIS UNIVERSITY SCHOOL OF MEDICINE	N01CM57020		14,841
CTEP	TENNESSEE UNIVERSITY CENTER FOR HEALTH SCIENCES	N01CM17472		87,299
CTEP	TEXAS, UNIVERSITY OF, SYSTEM CANCER CTR/MDA	N01CM07406		124,968
CTEP	TEXAS, UNIVERSITY OF	N01CM07417		58,782
CTEP	VETERANS ADMINISTRATION	Y01CM70107		200,000
CTEP	WAYNE STATE UNIVERSITY	N01CM07404		101,067
	TOTAL			3,229,134

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ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	PHASE IV CLINICAL TRIALS (COMBINED MODALITY):		
CTEP	AMERICAN COLLEGE OF RADIOLOGY	N01CM87219	135,546
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM07416	15,097
CTEP	CHICAGO, UNIVERSITY OF	N01CM07411	127,508
CTEP	CINCINNATI, UNIVERSITY OF	N01CM87222	33,302
CTEP	FRED HUTCHINSON CANCER RESEARCH CENTER	N01CM07336	30,678
CTEP	HEALTH RESEARCH, INC.	N01CM07410	145,316
CTEP	ILLINOIS CANCER COUNCIL	N01CM07415	22,898
CTEP	ISTITUTO NAZIONALE TUMORI	N01CM07338	72,750
CTEP	ISTITUTO NAZIONALE TUMORI	N01CM43726	90,360
CTEP	MARYLAND, UNIVERSITY OF	N01CM87223	21,209
CTEP	MAYO FOUNDATION	N01CM07414	26,160
CTEP	MAYO FOUNDATION	N01CM43783	19,602
CTEP	MAYO FOUNDATION	N01CM43796	30,690
CTEP	MEMORIAL HOSP. FOR CANCER & ALLIED DISEASES	N01CM87224	15,050
CTEP	MIAMI, UNIVERSITY OF	N01CM07409	142,460
CTEP	MICHIGAN, UNIVERSITY OF	N01CM87225	57,500
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM07407	138,295
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	10,530
CTEP	NORTHERN CALIFORNIA CANCER PROGRAM	N01CM87154	7,251
CTEP	ONTARIO CANCER INSTITUTE	N01CM07418	47,821
CTEP	PITTSBURGH, UNIVERSITY OF	N01CM77177	800,282
CTEP	SOUTH FLORIDA, UNIVERSITY OF	N01CM87220	64,328
CTEP	TEXAS, UNIVERSITY OF	N01CM07417	19,594
CTEP	TEXAS, UNIVERSITY OF, MEDICAL BRANCH	N01CM87221	40,250

ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
PHASE IV CLINICAL TRIALS (COMBINED MODALITY):			
CTEP	VETERANS ADMINISTRATION	Y01CM70107	200,000
CTEP	WAYNE STATE UNIVERSITY	N01CM07408	87,224
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM07339	167,979
TOTAL			2,569,680

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ANALYSIS OF CONTRACTS BY ACTIVITY

ACTIVITY		FOR FISCAL YEAR 1981 AS OF 05/31/81	
AREA	CONTRACT	#	DOLLAR LEVEL
CLINICAL TRIALS - OTHER RESEARCH:			
CTEP	CALIFORNIA, UNIVERSITY OF	N01CM97315	211,006
CTEP	FOX CHASE CANCER CENTER	N01CM97314	439,448
CTEP	HEALTH RESEARCH, INC.	N01CM97311	2,774
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N01CM17349	51,525
CTEP	MAYO FOUNDATION	N01CM43783	6,534
CTEP	MAYO FOUNDATION	N01CM43796	12,276
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y01CM00103	5,854
CTEP	VETERANS ADMINISTRATION	Y01CM70107	100,000
CTEP	WASHINGTON, UNIVERSITY OF	N01CM97282	279,760
TOTAL			1,109,177

ANALYSIS OF CONTRACTS BY ACTIVITY

AREA	ACTIVITY CONTRACT	FOR FISCAL YEAR 1981 AS OF 05/31/81	DOLLAR LEVEL
	CLINICAL TRIALS SUPPORTIVE RESEARCH:		
CTEP	CHICAGO, UNIVERSITY OF	N01CM07411	14,168
CTEP	EMMES CORPORATION	N01CM87193	32,172
CTEP	GEORGETOWN UNIVERSITY	N01CM87194	118,565
CTEP	HEALTH RESEARCH, INC.	N01CM07410	16,146
CTEP	INFORMATION MANAGEMENT SERVICES, INC.	N01CP01025	100,000
CTEP	MATHTECH, INC.	N01CM97195	550,972
CTEP	MIAMI, UNIVERSITY OF	N01CM07409	15,829
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM07407	15,366
CTEP	MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096	1,170
C.O.P.	NATIONAL NAVAL MEDICAL CENTER	Y01CM00103	6,059
D.T.P.	OHIO STATE UNIVERSITY RESEARCH FOUNDATION	N01CM17375	78,307
CTEP	WAYNE STATE UNIVERSITY	N01CM07408	9,692
CTEP	YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM07339	18,664
	TOTAL		977,110

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TABLE III
ANALYSIS OF ACTIVITIES BY CONTRACTS
FOR FISCAL YEAR 1981
AS OF 05/31/81

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
AGRICULTURE, DEPARTMENT OF	Y01CM40001 D.T.P.		451,154
ACQUISITION OF MATERIALS		* 90.00*	406,039
NEW AGENT PROCUREMENT, PLANT PRODUCTS		85.00	383,481
DATA PROCESSING AND SUPPORT		5.00	22,558
PROCUREMENT OF PRECLINICAL MATERIAL PLANT PRODUCTS		* 10.00*	45,115
ALABAMA, UNIVERSITY OF	N01CP95616B A.P.		15,000
ACQUISITION OF MATERIALS		* 20.00*	3,000
ANIMALS			
BASIC SCREEN		* 55.00*	8,250
ANIMALS			
VERIFICATION SCREEN		* 25.00*	3,750
ANIMALS			
ALABAMA, UNIVERSITY OF	N01CM07355 D.T.P.		17,461
ACQUISITION OF MATERIALS		* 100.00*	17,461
NEW AGENT PROCUREMENT, SYNTHETICS			
ALBERT EINSTEIN SCHOOL OF MEDICINE	N01CM17340 CTEP		171,666
PHASE I CLINICAL TRIALS		* 20.00*	34,333
UTERINE CERVIX CHEM.			
PHASE II CLINICAL TRIALS		* 20.00*	34,333
UTERINE CERVIX CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	103,000
UTERINE CERVIX CHEM.			
ALDRICH CHEMICAL COMPANY, INC.	N01CM17492 D.T.P.		305,635
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	152,818
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	152,818
PRODUCTION, SYNTHETICS			
AMERICAN COLLEGE OF RADIOLOGY	N01CM87219 CTEP		135,546
PHASE IV CLINICAL TRIALS		* 100.00*	135,546
HEAD AND NECK		30.00	40,664
HEAD AND NECK SURG.		30.00	40,664
HEAD AND NECK RAD.			

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
HEAD AND NECK	CHEM.	20.00	27,109
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	27,109
AMERICAN TYPE CULTURE COLLECTION	N01CM05725 D.T.P.		80,720
PROCUREMENT OF PRECLINICAL MATERIAL TISSUE CULTURE		*100.00*	80,720
ARIZONA STATE UNIVERSITY	N01CM17497 D.T.P.		272,850
BASIC SCREEN		* 90.00*	245,565
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	27,285
DETAILED DRUG EVALUATION, IN VITRO			
ARIZONA STATE UNIVERSITY	N01CM97262 D.T.P.		128,000
ACQUISITION OF MATERIALS		*100.00*	128,000
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS			
ARIZONA STATE UNIVERSITY	N01CM97297 D.T.P.		168,683
ACQUISITION OF MATERIALS		* 85.00*	143,381
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	109,644
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	25,302
BIOASSAY OF NATURAL PRODUCTS		5.00	8,434
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	25,302
PLANT PRODUCTS		10.00	16,868
FERMENTATION/ANTIBIOTICS		5.00	8,434
ARIZONA, UNIVERSITY OF	N01CM17500 CTEP		116,209
PHASE I CLINICAL TRIALS		* 20.00*	23,242
UTERINE CERVIX	CHEM.		
PHASE II CLINICAL TRIALS		* 20.00*	23,242
UTERINE CERVIX	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	69,725
UTERINE CERVIX	CHEM.		
ARTHUR D. LITTLE, INC.	N01CM07257B CTEP		194,058
BASIC SCREEN		* 30.00*	58,217
PRIMARY SCREENING, IN VITRO		10.00	19,406
PRIMARY SCREENING, IN VIVO		10.00	19,406
PHYS/CHEM PROPS.		10.00	19,406
VERIFICATION SCREEN		* 70.00*	135,841
DETAILED DRUG EVALUATION, IN VIVO			
ARTHUR D. LITTLE, INC.	N01CM07302 D.T.P.		487,456
BASIC SCREEN		* 55.00*	268,101

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
IN VIVO ANALOG SCR.			
TREATMENT STUDIES		* 45.00*	219,355
COMB. MODAL. THERAPY		45.00	219,355
COMB. MODAL. THERAPY - RAD.		20.00	97,491
COMB. MODAL. THERAPY - CHEM.		25.00	121,864
ARTHUR D. LITTLE, INC.	N01CM07331 D.T.P.		112,589
* BASIC SCREEN		* 94.00*	105,834
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 6.00*	6,755
DETAILED DRUG EVALUATION, IN VITRO		3.00	3,378
DETAILED DRUG EVALUATION, IN VIVO		3.00	3,378
ARTHUR D. LITTLE, INC.	N01CM07346 D.T.P.		525,603
ACQUISITION OF MATERIALS		* 15.00*	78,840
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 55.00*	289,082
PRIMARY SCREENING, IN VIVO		50.00	262,802
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	26,280
VERIFICATION SCREEN		* 25.00*	131,401
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 5.00*	26,280
CHEMOTHERAPY			
ARTHUR D. LITTLE, INC.	N01CM87163 D.T.P.		459,242
PHARMACOLOGY/TOXICOLOGY		*100.00*	459,242
PHARMACOLOGY		80.00	367,394
RADIOAUTOG/ANAL		20.00	91,848
ARTHUR D. LITTLE, INC.	N01CM97288 D.T.P.		94,534
ACQUISITION OF MATERIALS		* 11.00*	10,399
STRUCTURE ACTIVITY			
CELLULAR/SUBCELLULAR STUDIES		* 62.00*	58,611
BLOOD PRODUCTS		12.00	11,344
CELL KINETICS		15.00	14,180
MOLECULAR BIOLOGY		20.00	18,907
OTHER		15.00	14,180
PROGRAM MANAGEMENT		* 15.00*	14,180
ADMINISTRATION			
PHASE I CLINICAL TRIALS		* 12.00*	11,344
PANCREATIC PHARM./TOX.			
ASH STEVENS, INC.	N01CM17488 D.T.P.		437,448
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	218,724
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	218,724

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PRODUCTION, SYNTHETICS			
BATTELLE MEMORIAL INSTITUTE	N01CM07266 D.T.P.		940,825
ACQUISITION OF MATERIALS		* 15.00*	141,124
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 55.00*	517,454
PRIMARY SCREENING, IN VIVO		50.00	470,413
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	47,041
VERIFICATION SCREEN		* 25.00*	235,206
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 5.00*	47,041
CHEMOTHERAPY			
BATTELLE MEMORIAL INSTITUTE	N01CM17365 D.T.P.		3,281,708
PHARMACOLOGY/TOXICOLOGY		*100.00*	3,281,708
PROTOCOL TOXICITY STUDIES		50.00	1,640,854
SPECIAL TOXICITY STUDIES		34.00	1,115,781
ANIMALS		1.00	32,817
DATA PROCESSING AND SUPPORT		15.00	492,256
BATTELLE MEMORIAL INSTITUTE	N01CM67099 D.T.P.		520,930
BASIC SCREEN		*100.00*	520,930
PRIMARY SCREENING, IN VIVO		75.00	390,698
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		25.00	130,233
BEN VENUE LABORATORIES, INC.	N01CM97298 D.T.P.		856,765
FORMULATION		* 10.00*	85,677
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	771,089
FORMULATION		75.00	642,574
ANALYTICAL AND QUALITY CONTROL		15.00	128,515
BOWMAN GRAY SCHOOL OF MEDICINE OF WAKE	N01CM67054 CTEP		12,058
PROGRAM MANAGEMENT		* 10.00*	1,206
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	3,617
CNS TUMORS CHEM.			
PHASE III CLINICAL TRIALS		* 60.00*	7,235
CNS TUMORS RAD.		30.00	3,617
CNS TUMORS CHEM.		30.00	3,617
BRISTOL LABORATORIES	N01CM07299 D.T.P.		524,763
ACQUISITION OF MATERIALS		* 85.00*	446,049
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	435,553
DATA PROCESSING AND SUPPORT		2.00	10,495

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
BASIC SCREEN		* 15.00*	78,714
PRIMARY SCREENING, IN VITRO		10.00	52,476
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	26,238
BRISTOL LABORATORIES	N01CM07324 D.T.P.		221,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		*100.00*	221,000
CALIFORNIA, UNIVERSITY OF	N01CM07416 CTEP		75,484
PHASE II CLINICAL TRIALS		* 20.00*	15,097
LUNG RAD.		10.00	7,548
LUNG CHEM.		10.00	7,548
PHASE III CLINICAL TRIALS		* 60.00*	45,290
LUNG RAD.		30.00	22,645
LUNG CHEM.		30.00	22,645
PHASE IV CLINICAL TRIALS		* 20.00*	15,097
LUNG RAD.		10.00	7,548
LUNG CHEM.		10.00	7,548
CALIFORNIA, UNIVERSITY OF	N01CM97315 CTEP		211,006
OTHER CLINICAL TRIALS RESEARCH NON-SPECIFIC HARDWARE DEVEL.		*100.00*	211,006
CALIFORNIA, UNIVERSITY OF	N01CM07420 D.T.P.		241,500
BASIC SCREEN		* 90.00*	217,350
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	24,150
DETAILED DRUG EVALUATION, IN VITRO			
CANCER THERAPY & RESEARCH FOUND. OF	N01CM07327 D.T.P.		347,292
BASIC SCREEN		* 90.00*	312,563
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	34,729
DETAILED DRUG EVALUATION, IN VITRO			
CDP ASSOCIATES	N01CM97143 O.D.		311,867
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	311,867
CHARLES RIVER BREEDING LABS.	N01CM50598 A.P.		249,210
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	49,842
BASIC SCREEN ANIMALS		* 55.00*	137,066

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
VERIFICATION SCREEN ANIMALS		* 25.00*	62,303
CHARLES RIVER BREEDING LABS.	N01CM90163 A.P.		237,510
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	47,502
BASIC SCREEN ANIMALS		* 55.00*	130,631
VERIFICATION SCREEN ANIMALS		* 25.00*	59,378
CHARLES RIVER BREEDING LABS.	N01CM17498 A.P.		150,540
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	30,108
BASIC SCREEN ANIMALS		* 55.00*	82,797
VERIFICATION SCREEN ANIMALS		* 25.00*	37,635
CHARLES RIVER BREEDING LABS.	N01CM77141 A.P.		519,693
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	103,939
BASIC SCREEN ANIMALS		* 55.00*	285,831
VERIFICATION SCREEN ANIMALS		* 25.00*	129,923
CHARLES RIVER BREEDING LABS.	N01CM97229 A.P.		369,640
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	73,928
BASIC SCREEN ANIMALS		* 55.00*	203,302
VERIFICATION SCREEN ANIMALS		* 25.00*	92,410
CHEMICAL ABSTRACTS SERVICE	N01CM43722 D.T.P.		522,000
ACQUISITION OF MATERIALS DATA PROCESSING AND SUPPORT		* 100.00*	522,000
CHICAGO, UNIVERSITY OF	N01CM07411 CTEP		141,676
PHASE IV CLINICAL TRIALS		* 90.00*	127,508
STOMACH SURG.		25.00	35,419
STOMACH RAD.		25.00	35,419
STOMACH CHEM.		40.00	56,670
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	14,168

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
STOMACH	MARKERS		
CINCINNATI, UNIVERSITY OF	N01CM87222 CTEP		33,302
PHASE IV CLINICAL TRIALS		*100.00*	33,302
HEAD AND NECK	SURG.	30.00	9,991
HEAD AND NECK	RAD.	30.00	9,991
HEAD AND NECK	CHEM.	20.00	6,660
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	6,660
CLINICA NEUROCHIRURGICA DELL	N01CM67056 CTEP		10,000
PROGRAM MANAGEMENT		* 10.00*	1,000
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	3,000
CNS TUMORS	RAD.		
PHASE III CLINICAL TRIALS		* 60.00*	6,000
CNS TUMORS	RAD.	30.00	3,000
CNS TUMORS	CHEM.	30.00	3,000
COLLABORATIVE RESEARCH, INC.	N01CM07358 D.T.P.		14,984
ACQUISITION OF MATERIALS		*100.00*	14,984
NEW AGENT PROCUREMENT, SYNTHETICS			
CORDOVA CHEMICAL COMPANY	N01CM17490 D.T.P.		397,096
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	198,548
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	198,548
PRODUCTION, SYNTHETICS			
DUKE UNIVERSITY	N01CM17477 CTEP		43,082
PROGRAM MANAGEMENT		* 10.00*	4,308
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	12,925
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	25,849
CNS TUMORS	RAD.	30.00	12,925
CNS TUMORS	CHEM.	30.00	12,925
DUKE UNIVERSITY	N01CM67010 CTEP		15,000
PROGRAM MANAGEMENT		* 10.00*	1,500
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	4,500
CNS TUMORS	RAD.		
PHASE III CLINICAL TRIALS		* 60.00*	9,000
CNS TUMORS	RAD.	30.00	4,500
CNS TUMORS	CHEM.	30.00	4,500

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
EMMES CORPORATION	N01CM87193		32,172
CLINICAL TRIALS SUPPORTIVE RESEARCH	CTEP		
LUNG DATA PROC. & SUPP.		*100.00*	32,172
ENVIRO CONTROL, INC.	N01CM07332		94,887
ACQUISITION OF MATERIALS	D.T.P.		
LIT. MONITOR.		*100.00*	94,887
FLOW LABORATORIES, INC.	N01CM67088		115,452
PROD. AND FORM. FOR CLINICAL TRIALS	D.T.P.		
STOR.&DIST. OF DRUGS		*100.00*	115,452
FLOW LABORATORIES, INC.	N01CM97254		376,107
ACQUISITION OF MATERIALS	D.T.P.		
DATA PROCESSING AND SUPPORT		* 60.00*	225,664
STORAGE DISTRIBUTION		10.00	37,611
PROCUREMENT OF PRECLINICAL MATERIAL		50.00	188,054
STORAGE DISTRIBUTION		* 30.00*	112,832
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	37,611
DATA PROCESSING AND SUPPORT		1.00	3,761
STORAGE DISTRIBUTION		9.00	33,850
FOX CHASE CANCER CENTER	N01CM07330B		90,269
BASIC SCREEN	CTEP		
PRIMARY SCREENING, IN VIVO		* 80.00*	72,215
VERIFICATION SCREEN		* 20.00*	18,054
DETAILED DRUG EVALUATION, IN VIVO			
FOX CHASE CANCER CENTER	N01CM97314		439,448
OTHER CLINICAL TRIALS RESEARCH	CTEP		
NON-SPECIFIC HARDWARE DEVEL.		*100.00*	439,448
FRED HUTCHINSON CANCER RESEARCH	N01CM07336		153,391
PHASE II CLINICAL TRIALS	CTEP		
LUNG RAD.		* 20.00*	30,678
LUNG CHEM.		10.00	15,339
PHASE III CLINICAL TRIALS		10.00	15,339
LUNG RAD.		* 60.00*	92,035
LUNG CHEM.		30.00	46,017
PHASE IV CLINICAL TRIALS		30.00	46,017
LUNG RAD.		* 20.00*	30,678
LUNG CHEM.		10.00	15,339
		10.00	15,339

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
GEORGETOWN UNIVERSITY	N01CM17501		98,974
CTEP			
PHASE I CLINICAL TRIALS		* 20.00*	19,795
UTERINE CERVIX	CHEM.		
PHASE II CLINICAL TRIALS		* 20.00*	19,795
UTERINE CERVIX	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	59,384
UTERINE CERVIX	CHEM.		
GEORGETOWN UNIVERSITY	N01CM87194		118,565
CTEP			
CLINICAL TRIALS SUPPORTIVE RESEARCH	* 100.00*	118,565	
NON-SPECIFIC	OTHER		
GEORGETOWN UNIVERSITY	N01CM97208		173,508
CTEP			
PHASE I CLINICAL TRIALS	* 71.00*	123,191	
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS	* 29.00*	50,317	
LEUK.-ACUTE GRAN.	CHEM.	2.00	3,470
LEUK.-ACUTE LYMPH.	CHEM.	2.00	3,470
GENERAL	CHEM.	5.00	8,675
BREAST	CHEM.	5.00	8,675
COLORRECTAL	CHEM.	5.00	8,675
LUNG	CHEM.	5.00	8,675
MELANOMA	CHEM.	5.00	8,675
HARLAN INDUSTRIES	N01CM50591		219,960
A.P.			
ACQUISITION OF MATERIALS	* 20.00*	43,992	
ANIMALS			
BASIC SCREEN	* 55.00*	120,978	
ANIMALS			
VERIFICATION SCREEN	* 25.00*	54,990	
ANIMALS			
HARLAN INDUSTRIES	N01CM07362		557,413
A.P.			
ACQUISITION OF MATERIALS	* 20.00*	111,483	
ANIMALS			
BASIC SCREEN	* 55.00*	306,577	
ANIMALS			
VERIFICATION SCREEN	* 25.00*	139,353	
ANIMALS			
HARLAN INDUSTRIES	N01CM97242		275,830
A.P.			
ACQUISITION OF MATERIALS	* 20.00*	55,166	
ANIMALS			
BASIC SCREEN	* 55.00*	151,707	

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS VERIFICATION SCREEN ANIMALS		* 25.00*	68,958
HARLAN INDUSTRIES	N01CM97243 A.P.		200,580
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	40,116
BASIC SCREEN ANIMALS		* 55.00*	110,319
VERIFICATION SCREEN ANIMALS		* 25.00*	50,145
HAZELTON LABGRATORIES, INC.	N01CM15770 C.O.P.		110,000
CELLULAR/SUBCELLULAR STUDIES		*100.00*	110,000
BLOOD PRODUCTS		50.00	55,000
IMMUNOBIOLOGY		50.00	55,000
HAZLETON LABORATORIES, INC.	N01CM60125 A.P.		35,000
PHARMACOLOGY/TOXICOLOGY ANIMALS		*100.00*	35,000
HAZLETON LABORATORIES, INC.	N01CM87156 C.O.P.		145,000
CELLULAR/SUBCELLULAR STUDIES		* 70.00*	101,500
BIOCHEMISTRY		20.00	29,000
CELL KINETICS		20.00	29,000
MARKERS		10.00	14,500
NUTRITION		20.00	29,000
TREATMENT STUDIES		* 30.00*	43,500
SURGERY		10.00	14,500
RADIO THERAPY		10.00	14,500
CHEMOTHERAPY		10.00	14,500
HAZLETON LABORATORIES, INC.	N01CM97217 D.T.P.		479,670
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	95,934
VERIFICATION SCREEN ANIMALS		* 10.00*	47,967
PHARMACOLOGY/TOXICOLOGY		* 40.00*	191,868
PHARMACOLOGY		10.00	47,967
SPECIAL TOXICITY STUDIES		30.00	143,901
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	143,901
MARKERS		30.00	143,901
HEALTH RESEARCH, INC.	N01CM97311 CTEP		2,774
OTHER CLINICAL TRIALS RESEARCH		*100.00*	2,774

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
NON-SPECIFIC PHOTORADIATION THE			
HEALTH RESEARCH, INC.	N01CM77101 A.P.		3,784
ACQUISITION OF MATERIALS		* 20.00*	757
ANIMALS			
BASIC SCREEN		* 55.00*	2,081
ANIMALS			
VERIFICATION SCREEN		* 25.00*	946
ANIMALS			
HEALTH RESEARCH, INC.	N01CM07410 CTEP		161,462
PHASE IV CLINICAL TRIALS		* 90.00*	145,316
STOMACH		25.00	40,366
STOMACH	SURG.	25.00	40,366
STOMACH	RAD.	40.00	64,585
STOMACH	CHEM.		16,146
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	
STOMACH	MARKERS		
IIT RESEARCH INSTITUTE	N01CM97213 D.T.P.		299,450
ACQUISITION OF MATERIALS		* 20.00*	59,890
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 65.00*	194,643
PRIMARY SCREENING, IN VITRO		5.00	14,973
PRIMARY SCREENING, IN VIVO		55.00	164,698
DATA PROCESSING AND SUPPORT		5.00	14,973
VERIFICATION SCREEN		* 15.00*	44,918
DETAILED DRUG EVALUATION, IN VIVO			
IIT RESEARCH INSTITUTE	N01CM97316 D.T.P.		973,378
ACQUISITION OF MATERIALS		* 15.00*	146,007
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 71.00*	691,098
PRIMARY SCREENING, IN VIVO		64.00	622,962
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		7.00	68,136
VERIFICATION SCREEN		* 11.00*	107,072
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 3.00*	29,201
CHEMOTHERAPY			
ILLINOIS CANCER COUNCIL	N01CM07415 CTEP		114,483
PHASE II CLINICAL TRIALS		* 20.00*	22,898
LUNG		10.00	11,449
LUNG	RAD.	10.00	11,449
LUNG	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	68,693
LUNG		30.00	34,346
LUNG	RAD.	30.00	34,346
LUNG	CHEM.		

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PHASE IV CLINICAL TRIALS		* 20.00*	22,898
LUNG	RAD.	10.00	11,449
LUNG	CHEM.	10.00	11,449
ILLINOIS, UNIVERSITY OF	N01CM97259 D.T.P.		99,837
ACQUISITION OF MATERIALS LITERATURE SURVEILL.		*100.00*	99,837
ILLINOIS, UNIVERSITY OF	N01CM97295 D.T.P.		154,564
ACQUISITION OF MATERIALS		* 85.00*	131,379
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	100,467
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	23,185
BIOASSAY OF NATURAL PRODUCTS		5.00	7,728
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	23,185
PLANT PRODUCTS		10.00	15,456
FERMENTATION/ANTIBIOTICS		5.00	7,728
INDIANA UNIVERSITY FOUNDATION	N01CM17475 CTEP		58,597
PROGRAM MANAGEMENT		* 10.00*	5,860
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	17,579
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	35,158
CNS TUMORS	RAD.	30.00	17,579
CNS TUMORS	CHEM.	30.00	17,579
INFORMATION MANAGEMENT SERVICES,	N01CM17349 CTEP		57,250
PROGRAM MANAGEMENT		* 10.00*	5,725
ADMINISTRATION			
OTHER CLINICAL TRIALS RESEARCH		* 90.00*	51,525
CNS TUMORS	DATA PROC. & SUPP.		
INFORMATION MANAGEMENT SERVICES,	N01CP01025 CTEP		100,000
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	100,000
LUNG	DATA PROC. & SUPP.		
INFORMATION PLANNING ASSOC.	N01CM77104 CTEP		208,979
PROGRAM MANAGEMENT		*100.00*	208,979
DATA PROCESSING AND SUPPORT			
INSTITUT JULES BORDET	N01CM07350 D.T.P.		199,286
BASIC SCREEN		* 80.00*	159,429
PRIMARY SCREENING, IN VIVO			
VERIFICATION SCREEN		* 20.00*	39,857

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
DETAILED DRUG EVALUATION, IN VIVO			
INSTITUT JULES BORDET	N01CM53840 O.D.		44,800
ACQUISITION OF MATERIALS	* 30.00*	13,440	
NEW AGENT PROCUREMENT, SYNTHETICS	25.00	11,200	
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT	5.00	2,240	
CELLULAR/SUBCELLULAR STUDIES	* 20.00*	8,960	
DRUG EVAL./INVEST.	20.00	8,960	
PROGRAM MANAGEMENT	* 50.00*	22,400	
COMMUNICATION AND EDUCATION	30.00	13,440	
RESOURCE DEVELOPMENT	5.00	2,240	
DATA PROCESSING AND SUPPORT	15.00	6,720	
INSTITUTE OF CANCER RESEARCH	N01CM77139B CTEP		17,000
ACQUISITION OF MATERIALS	* 100.00*	17,000	
NEW AGENT PROCUREMENT, SYNTHETICS			
INSTITUTE OF CANCER RESEARCH	N01CM43736 D.T.P.		105,000
ACQUISITION OF MATERIALS	* 50.00*	52,500	
NEW AGENT PROCUREMENT, SYNTHETICS	40.00	42,000	
DATA PROCESSING AND SUPPORT	10.00	10,500	
VERIFICATION SCREEN	* 50.00*	52,500	
DETAILED DRUG EVALUATION, IN VITRO	5.00	5,250	
DETAILED DRUG EVALUATION, IN VIVO	20.00	21,000	
DET. DRUG EVAL., RELATED NEW MODEL DEVEL	25.00	26,250	
IOWA, UNIVERSITY OF	N01CM07334 BRMP		105,244
PROCUREMENT OF PRECLINICAL MATERIAL	* 70.00*	73,671	
ANALYTICAL AND QUALITY CONTROL			
PROD. AND FORM. FOR CLINICAL TRIALS	* 30.00*	31,573	
ANALYTICAL AND QUALITY CONTROL			
IOWA, UNIVERSITY OF	N01CM17476 CTEP		90,476
PROGRAM MANAGEMENT	* 10.00*	9,048	
ADMINISTRATION			
PHASE II CLINICAL TRIALS	* 30.00*	27,143	
CNS TUMORS CHEM.			
PHASE III CLINICAL TRIALS	* 60.00*	54,286	
CNS TUMORS RAD.	30.00	27,143	
CNS TUMORS CHEM.	30.00	27,143	
IOWA, UNIVERSITY OF	N01CM07303 D.T.P.		316,792
FORMULATION	* 20.00*	63,358	
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS	* 80.00*	253,434	

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
FORMULATION		70.00	221,754
ANALYTICAL AND QUALITY CONTROL		10.00	31,679
IOWA, UNIVERSITY OF	N01CM07412 D.T.P.		121,000
ACQUISITION OF MATERIALS		*100.00*	121,000
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
ISTITUTO NAZIONALE TUMORI	N01CM07338 CTEP		145,500
PHASE II CLINICAL TRIALS		* 15.00*	21,825
COLORECTAL	CHEM.	10.00	14,550
STOMACH	CHEM.	5.00	7,275
PHASE III CLINICAL TRIALS		* 35.00*	50,925
BREAST	CHEM.		
PHASE IV CLINICAL TRIALS		* 50.00*	72,750
BREAST	SURG.	16.00	23,280
BREAST	RAD.	6.00	8,730
BREAST	CHEM.	16.00	23,280
CNS TUMORS	SURG.	4.00	5,820
CNS TUMORS	RAD.	4.00	5,820
CNS TUMORS	CHEM.	4.00	5,820
ISTITUTO NAZIONALE TUMORI	N01CM43726 CTEP		150,600
PHASE II CLINICAL TRIALS		* 40.00*	60,240
MELANOMA	CHEM.		
PHASE IV CLINICAL TRIALS		* 60.00*	90,360
MELANOMA	SURG.	20.00	30,120
MELANOMA	CHEM.	20.00	30,120
MELANOMA	IMM.	20.00	30,120
JAPANESE FOUNDATION FOR CANCER RESEARCH	N01CM22054 O.D.		16,000
ACQUISITION OF MATERIALS		* 10.00*	1,600
NEW AGENT PROCUREMENT, SYNTHETICS		5.00	800
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		5.00	800
BASIC SCREEN		* 40.00*	6,400
PRIMARY SCREENING, IN VITRO		2.00	320
PRIMARY SCREENING, IN VIVO		18.00	2,880
DRUG EVALUATION		20.00	3,200
PROGRAM MANAGEMENT		* 20.00*	3,200
COMMUNICATION AND EDUCATION		10.00	1,600
RESOURCE DEVELOPMENT		10.00	1,600
PHASE I CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN.	CHEM.	5.00	800
LEUK.-ACUTE LYMPH.	CHEM.	5.00	800
NON-SPECIFIC	CHEM.	5.00	800
PHASE II CLINICAL TRIALS		* 15.00*	2,400
LEUK.-ACUTE GRAN.	CHEM.	5.00	800
LEUK.-ACUTE LYMPH.	CHEM.	5.00	800

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
NON-SPECIFIC CHEM.		5.00	800
KANSAS, UNIVERSITY OF	N01CM07304		75,235
FORMULATION DEVEL. OF EXP. FORMULATIONS	D.T.P.	*100.00*	75,235
KANSAS, UNIVERSITY OF-MEDICAL CENTER	N01CM97272		97,273
PHASE I CLINICAL TRIALS	CTEP	* 71.00*	69,064
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	28,209
LEUK.-ACUTE GRAN. CHEM.		2.00	1,945
LEUK.-ACUTE LYMPH. CHEM.		2.00	1,945
GENERAL CHEM.		5.00	4,864
BREAST CHEM.		5.00	4,864
COLORRECTAL CHEM.		5.00	4,864
LUNG CHEM.		5.00	4,864
MELANOMA CHEM.		5.00	4,864
KENTUCKY, UNIVERSITY OF	N01CM67058		12,000
PROGRAM MANAGEMENT	CTEP	* 10.00*	1,200
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	3,600
CNS TUMORS RAD.			
PHASE III CLINICAL TRIALS		* 60.00*	7,200
CNS TUMORS RAD.		30.00	3,600
CNS TUMORS CHEM.		30.00	3,600
KENTUCKY, UNIVERSITY OF	N01CM07381		75,945
FORMULATION	D.T.P.	*100.00*	75,945
DEVEL. OF EXP. FORMULATIONS			
KING ANIMAL LABORATORY	N01CM17499		143,520
ACQUISITION OF MATERIALS	A.P.	* 20.00*	28,704
ANIMALS			
BASIC SCREEN		* 55.00*	78,936
ANIMALS			
VERIFICATION SCREEN		* 25.00*	35,880
ANIMALS			
LABORATORY SUPPLY COMPANY, INC.	N01CM50577		224,640
ACQUISITION OF MATERIALS	A.P.	* 20.00*	44,928
ANIMALS			
BASIC SCREEN		* 55.00*	123,552
ANIMALS			
VERIFICATION SCREEN		* 25.00*	56,160

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
LABORATORY SUPPLY COMPANY, INC.	N01CM97244		208,975
	A.P.		
ACQUISITION OF MATERIALS		* 20.00*	41,795
ANIMALS			
BASIC SCREEN		* 55.00*	114,936
ANIMALS			
VERIFICATION SCREEN		* 25.00*	52,244
ANIMALS			
LEO GOODWIN INST. FOR CANCER RESEARCH	N01CM77165		803,629
	A.P.		
ACQUISITION OF MATERIALS		* 20.00*	160,726
ANIMALS			
BASIC SCREEN		* 55.00*	441,996
ANIMALS			
VERIFICATION SCREEN		* 25.00*	200,907
ANIMALS			
LITTON BIONETICS, INC.	N01CM67067		200,000
	C.O.P.		
PROD. AND FORM. FOR CLINICAL TRIALS		* 10.00*	20,000
DATA PROCESSING AND SUPPORT			
CELLULAR/SUBCELLULAR STUDIES		* 90.00*	180,000
IMMUNOLOGY		90.00	180,000
LITTON BIONETICS, INC.	N01CM05724		425,000
	D.T.P.		
PROCUREMENT OF PRECLINICAL MATERIAL		*100.00*	425,000
ANIMAL FACILITIES			
LITTON BIONETICS, INC.	N01CM07326		149,400
	D.T.P.		
ACQUISITION OF MATERIALS		*100.00*	149,400
ANIMAL VIRUSES			
LITTON BIONETICS, INC.	N01CM07347		498,000
	D.T.P.		
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	249,000
ANTIBODIES/ANTIGENS			
CELLULAR/SUBCELLULAR STUDIES		* 50.00*	249,000
MOLECULAR BIOLOGY		50.00	249,000
LITTON BIONETICS, INC.	N01CM87187		261,880
	D.T.P.		
CELLULAR/SUBCELLULAR STUDIES		*100.00*	261,880
BIOCHEMISTRY		30.00	78,564
CELL BIOLOGY		30.00	78,564
IMMUNOBIOLOGY		10.00	26,188
MOLECULAR BIOLOGY		30.00	78,564

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LITTON BIONETICS, INC.	N01C075380D D.T.P.		3,540,000
ACQUISITION OF MATERIALS		* 48.00*	1,699,200
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT			
BASIC SCREEN		* 12.00*	424,800
PRIMARY SCREENING, IN VITRO		3.00	106,200
ANIMALS		9.00	318,600
VERIFICATION SCREEN		* 30.00*	1,062,000
ANIMALS			
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	354,000
FERMENTATION/ANTIBIOTICS			
MARSHALL RESEARCH ANIMALS, INC.	N01CM60123 A.P.		25,250
PHARMACOLOGY/TOXICOLOGY		*100.00*	25,250
ANIMALS			
MARYLAND, UNIVERSITY OF	N01CM87223 CTEP		21,209
PHASE IV CLINICAL TRIALS		*100.00*	21,209
HEAD AND NECK	SURG.	30.00	6,363
HEAD AND NECK	RAD.	30.00	6,363
HEAD AND NECK	CHEM.	20.00	4,242
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	4,242
MASON RESEARCH INSTITUTE	N01CM87164 A.P.		198,624
BASIC SCREEN		* 8.00*	15,890
TUMOR BANK			
PHARMACOLOGY/TOXICOLOGY		* 77.00*	152,940
PHARMACOLOGY			
CELLULAR/SUBCELLULAR STUDIES		* 15.00*	29,794
BIOCHEMISTRY		15.00	29,794
MASON RESEARCH INSTITUTE	N01CM67011 C.O.P.		248,972
TREATMENT STUDIES		*100.00*	248,972
DATA PROCESSING AND SUPPORT			
MASON RESEARCH INSTITUTE/EG&G	N01CM07325 D.T.P.		202,922
VERIFICATION SCREEN		*100.00*	202,922
DET. DRUG EVAL., RELATED NEW MODEL DEVEL			
MASON RESEARCH INSTITUTE/EG&G	N01CM97317 D.T.P.		1,365,592
ACQUISITION OF MATERIALS		* 5.00*	68,280
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 61.00*	833,011
PRIMARY SCREENING, IN VIVO		50.00	682,796

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		11.00	150,215
VERIFICATION SCREEN		* 24.00*	327,742
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 10.00*	136,559
COMB. MODAL. THERAPY		10.00	136,559
COMB. MODAL. THERAPY - SURG.		8.00	109,247
COMB. MODAL. THERAPY - CHEM.		2.00	27,312
MATHTECH, INC.	N01CM97195		550,972
	CTEP		
CLINICAL TRIALS SUPPORTIVE RESEARCH		*100.00*	550,972
NON-SPECIFIC DATA PROC. & SUPP.			
MAYO FOUNDATION	N01CM07414		130,800
	CTEP		
PHASE II CLINICAL TRIALS		* 20.00*	26,160
LUNG RAD.		10.00	13,080
LUNG CHEM.		10.00	13,080
PHASE III CLINICAL TRIALS		* 60.00*	78,480
LUNG RAD.		30.00	39,240
LUNG CHEM.		30.00	39,240
PHASE IV CLINICAL TRIALS		* 20.00*	26,160
LUNG RAD.		10.00	13,080
LUNG CHEM.		10.00	13,080
MAYO FOUNDATION	N01CM43783		43,560
	CTEP		
PHASE III CLINICAL TRIALS		* 40.00*	17,424
STOMACH CHEM.			
PHASE IV CLINICAL TRIALS		* 45.00*	19,602
STOMACH SURG.		10.00	4,356
STOMACH RAD.		10.00	4,356
STOMACH CHEM.		25.00	10,890
OTHER CLINICAL TRIALS RESEARCH		* 15.00*	6,534
STOMACH DATA PROC. & SUPP.			
MAYO FOUNDATION	N01CM43796		61,380
	CTEP		
PHASE II CLINICAL TRIALS		* 30.00*	18,414
PANCREATIC CHEM.			
PHASE IV CLINICAL TRIALS		* 50.00*	30,690
PANCREATIC SURG.		5.00	3,069
PANCREATIC RAD.		20.00	12,276
PANCREATIC CHEM.		25.00	15,345
OTHER CLINICAL TRIALS RESEARCH		* 20.00*	12,276
PANCREATIC DATA PROC. & SUPP.			
MAYO FOUNDATION	N01CM57044		125,000
	CTEP		
PHASE II CLINICAL TRIALS		* 55.00*	68,750
BREAST CHEM.		15.00	18,750

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COLORECTAL	CHEM.	15.00	18,750
HEAD AND NECK	CHEM.	5.00	6,250
LUNG	CHEM.	5.00	6,250
MELANOMA	CHEM.	10.00	12,500
SARCOMAS (GEN.)	CHEM.	5.00	6,250
PHASE III CLINICAL TRIALS		* 45.00*	56,250
BREAST	CHEM.	10.00	12,500
COLORECTAL	CHEM.	5.00	6,250
HEAD AND NECK	CHEM.	5.00	6,250
LUNG	CHEM.	5.00	6,250
MELANOMA	CHEM.	15.00	18,750
SARCOMAS (GEN.)	CHEM.	5.00	6,250
MAYO FOUNDATION	N01CM97273 CTEP		256,064
PHASE I CLINICAL TRIALS		* 71.00*	181,805
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS		* 29.00*	74,259
LEUK.-ACUTE GRAN.	CHEM.	2.00	5,121
LEUK.-ACUTE LYMPH.	CHEM.	2.00	5,121
GENERAL	CHEM.	5.00	12,803
BREAST	CHEM.	5.00	12,803
COLORECTAL	CHEM.	5.00	12,803
LUNG	CHEM.	5.00	12,803
MELANOMA	CHEM.	5.00	12,803
MAYO FOUNDATION	N01CM07419 D.T.P.		205,049
BASIC SCREEN		* 90.00*	184,544
PRIMARY SCREENING, IN VITRO			
VERIFICATION SCREEN		* 10.00*	20,505
DETAILED DRUG EVALUATION, IN VITRO			
MELOY LABORATORIES, INC.	N01CM07378 BRMP		56,000
ACQUISITION OF MATERIALS		* 100.00*	56,000
NEW AGENT PROCUREMENT, MARINE/ARTHROPODS			
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM07337 CTEP		245,318
PHASE II CLINICAL TRIALS		* 55.00*	134,925
BREAST	CHEM.	15.00	36,798
COLORECTAL	CHEM.	15.00	36,798
HEAD AND NECK	CHEM.	5.00	12,266
LUNG	CHEM.	5.00	12,266
MELANOMA	CHEM.	10.00	24,532
SARCOMAS (GEN.)	CHEM.	5.00	12,266
PHASE III CLINICAL TRIALS		* 45.00*	110,393
BREAST	CHEM.	10.00	24,532
COLORECTAL	CHEM.	5.00	12,266
HEAD AND NECK	CHEM.	5.00	12,266

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LUNG	CHEM.	5.00	12,266
MELANOMA	CHEM.	15.00	36,798
SARCOMAS (GEN.)	CHEM.	5.00	12,266
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM17348 CTEP		122,400
PROGRAM MANAGEMENT		* 10.00*	12,240
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	36,720
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	73,440
CNS TUMORS	RAD.	30.00	36,720
CNS TUMORS	CHEM.	30.00	36,720
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM87224 CTEP		15,050
PHASE IV CLINICAL TRIALS		*100.00*	15,050
HEAD AND NECK	SURG.	30.00	4,515
HEAD AND NECK	RAD.	30.00	4,515
HEAD AND NECK	CHEM.	20.00	3,010
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	3,010
MEMORIAL HOSP. FOR CANCER & ALLIED	N01CM97274 CTEP		174,827
PHASE I CLINICAL TRIALS		* 71.00*	124,127
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS		* 29.00*	50,700
LEUK.-ACUTE GRAN.	CHEM.	2.00	3,497
LEUK.-ACUTE LYMPH.	CHEM.	2.00	3,497
GENERAL	CHEM.	5.00	8,741
BREAST	CHEM.	5.00	8,741
COLORECTAL	CHEM.	5.00	8,741
LUNG	CHEM.	5.00	8,741
MELANOMA	CHEM.	5.00	8,741
MIAMI, UNIVERSITY OF	N01CM07409 CTEP		158,289
PHASE IV CLINICAL TRIALS		* 90.00*	142,460
STOMACH	SURG.	25.00	39,572
STOMACH	RAD.	25.00	39,572
STOMACH	CHEM.	40.00	63,316
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	15,829
STOMACH	MARKERS		
MIAMI, UNIVERSITY OF	N01CM97290 D.T.P.		175,129
ACQUISITION OF MATERIALS		* 11.00*	19,264
STRUCTURE ACTIVITY			
CELLULAR/SUBCELLULAR STUDIES		* 62.00*	108,580
BLOOD PRODUCTS		12.00	21,015
CELL KINETICS		15.00	26,269

BEST DOCUMENT AVAILABLE

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
MOLECULAR BIOLOGY		20.00	35,026
OTHER		15.00	26,269
PROGRAM MANAGEMENT		* 15.00*	26,269
ADMINISTRATION			
PHASE I CLINICAL TRIALS		* 12.00*	21,015
PANCREATIC PHARM./TOX.			
MICHIGAN TECHNOLOGICAL	N01CM07293		100,000
D.T.P.			
ACQUISITION OF MATERIALS		* 95.00*	95,000
NEW AGENT PROCUREMENT, SYNTHETICS			
BASIC SCREEN		* 5.00*	5,000
PRIMARY SCREENING, IN VITRO			
MICHIGAN, UNIVERSITY OF	N01CM07405		230,386
CTEP			
PHASE II CLINICAL TRIALS		* 55.00*	126,712
BREAST CHEM.		15.00	34,558
COLORECTAL CHEM.		15.00	34,558
HEAD AND NECK CHEM.		5.00	11,519
LUNG CHEM.		5.00	11,519
MELANOMA CHEM.		10.00	23,039
SARCOMAS (GEN.) CHEM.		5.00	11,519
PHASE III CLINICAL TRIALS		* 45.00*	103,674
BREAST CHEM.		10.00	23,039
COLORECTAL CHEM.		5.00	11,519
HEAD AND NECK CHEM.		5.00	11,519
LUNG CHEM.		5.00	11,519
MELANOMA CHEM.		15.00	34,558
SARCOMAS (GEN.) CHEM.		5.00	11,519
MICHIGAN, UNIVERSITY OF	N01CM87225		57,500
CTEP			
PHASE IV CLINICAL TRIALS		* 100.00*	57,500
HEAD AND NECK SURG.		30.00	17,250
HEAD AND NECK RAD.		30.00	17,250
HEAD AND NECK CHEM.		20.00	11,500
HEAD AND NECK PAT. SUPPORT. CARE		20.00	11,500
MICROBIOLOGICAL ASSOCIATES	N01CM97246		235,250
A.P.			
ACQUISITION OF MATERIALS		* 20.00*	47,050
ANIMALS			
BASIC SCREEN		* 55.00*	129,388
ANIMALS			
VERIFICATION SCREEN		* 25.00*	58,813
ANIMALS			
MICROBIOLOGICAL ASSOCIATES	N01CM97287B		275,625
A.P.			
ACQUISITION OF MATERIALS		* 20.00*	55,125

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
ANIMALS			
BASIC SCREEN		* 55.00*	151,594
ANIMALS			
VERIFICATION SCREEN		* 25.00*	68,906
ANIMALS			
MICROBIOLOGICAL ASSOCIATES	N01CM07369		100,000
	C.O.P.		
CELLULAR/SUBCELLULAR STUDIES		* 100.00*	100,000
IMMUNOBIOLOGY		50.00	50,000
SERUM STORAGE		50.00	50,000
MIDWEST RESEARCH INSTITUTE	N01CM87234		250,430
	D.T.P.		
PROCUREMENT OF PRECLINICAL MATERIAL		* 20.00*	50,086
ANALYTICAL AND QUALITY CONTROL			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	200,344
ANALYTICAL AND QUALITY CONTROL			
MISSOURI, UNIVERSITY OF	N01CM87157		111,945
	A.P.		
ACQUISITION OF MATERIALS		* 20.00*	22,389
ANIMALS			
BASIC SCREEN		* 55.00*	61,570
ANIMALS			
VERIFICATION SCREEN		* 25.00*	27,986
ANIMALS			
MISSOURI, UNIVERSITY OF	N01CM97211		90,265
	A.P.		
ACQUISITION OF MATERIALS		* 20.00*	18,053
ANIMALS			
BASIC SCREEN		* 55.00*	49,646
ANIMALS			
VERIFICATION SCREEN		* 25.00*	22,566
ANIMALS			
MONSANTO RESEARCH CORPORATION	N01CM97255		655,340
	D.T.P.		
PROCUREMENT OF PRECLINICAL MATERIAL		* 10.00*	65,534
PLANT PRODUCTS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 90.00*	589,806
PRODUCTION, SYNTHETICS		15.00	98,301
PRODUCTION, PLANT PRODUCTS		75.00	491,505
MONTEFIORE HOSPITAL	N01CM17474		104,322
	CTEP		
PROGRAM MANAGEMENT		* 10.00*	10,432
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	31,297
CNS TUMORS			
CHEM.			

BEST DOCUMENT AVAILABLE

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PHASE III CLINICAL TRIALS		* 60.00*	62,593
CNS TUMORS		30.00	31,297
CNS TUMORS		30.00	31,297
MOUNT SINAI SCHOOL OF MEDICINE	N01CM07407		153,661
	CTEP		
PHASE IV CLINICAL TRIALS		* 90.00*	138,295
STOMACH		25.00	38,415
STOMACH		25.00	38,415
STOMACH		40.00	61,464
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	15,366
STOMACH			
MOUNT SINAI SCHOOL OF MEDICINE	N01CM67096		11,700
	CTEP		
PHASE IV CLINICAL TRIALS		* 90.00*	10,530
COLORRECTAL		18.00	2,106
COLORRECTAL		18.00	2,106
COLORRECTAL		36.00	4,212
COLORRECTAL		18.00	2,106
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	1,170
COLORRECTAL			
MOUNT SINAI SCHOOL OF MEDICINE	N01CM97275		131,184
	CTEP		
PHASE I CLINICAL TRIALS		* 71.00*	93,141
NON-SPECIFIC			
PHASE II CLINICAL TRIALS		* 29.00*	38,043
LEUK.-ACUTE GRAN.		2.00	2,624
LEUK.-ACUTE LYMPH.		2.00	2,624
GENERAL		5.00	6,559
BREAST		5.00	6,559
COLORRECTAL		5.00	6,559
LUNG		5.00	6,559
MELANOMA		5.00	6,559
MOUNT SINAI SCHOOL OF MEDICINE	N01CM97294		76,000
	D.T.P.		
PHARMACOLOGY/TOXICOLOGY		* 10.00*	7,600
PHARMACOLOGY			
PHASE I CLINICAL TRIALS		* 80.00*	60,800
NON-SPECIFIC			
PHASE II CLINICAL TRIALS		* 10.00*	7,600
NON-SPECIFIC			
MURPHY BREEDING LABS., INC.	N01CM50579A		220,709
	A.P.		
ACQUISITION OF MATERIALS		* 20.00*	44,142
ANIMALS			
BASIC SCREEN		* 55.00*	121,390
ANIMALS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
VERIFICATION SCREEN ANIMALS		* 25.00*	55,177
NATIONAL ACADEMY OF SCIENCES	N01CM53850 A.P.		27,000
PROGRAM MANAGEMENT COMMUNICATION AND EDUCATION		*100.00*	27,000
NATIONAL NAVAL MEDICAL CENTER	Y01CM00103 C.O.P.		68,075
PROGRAM MANAGEMENT		* 2.00*	1,362
COMMUNICATION AND EDUCATION		1.00	681
RESOURCE DEVELOPMENT		1.00	681
PHASE II CLINICAL TRIALS		* 80.50*	54,800
LEUK.-ACUTE GRAN.	CHEM.	1.00	681
LEUK.-ACUTE GRAN.	PAT. SUPPORT. CARE	1.00	681
LEUK.-ACUTE LYMPH.	CHEM.	1.50	1,021
LEUK.-ACUTE LYMPH.	IMM.	0.30	204
LEUK.-ACUTE LYMPH.	PAT. SUPPORT. CARE	1.50	1,021
LEUK.-CHRON. GRAN.	CHEM.	0.50	340
LEUK.-CHRON. GRAN.	PAT. SUPPORT. CARE	0.20	136
LEUK.-CHRON LYMPH.	CHEM.	0.50	340
LEUK.-CHRON LYMPH.	PAT. SUPPORT. CARE	0.50	340
LYMPHOMA-HISTIOCYT	CHEM.	0.50	340
LYMPHOMA-HISTIOCYT	PAT. SUPPORT. CARE	0.50	340
LYMPHOMA-HODGKIN'S	RAD.	1.00	681
LYMPHOMA-HODGKIN'S	CHEM.	1.00	681
LYMPHOMA-HODGKIN'S	PHARM./TOX.	0.50	340
LYMPHOMA-HODGKIN'S	PAT. SUPPORT. CARE	1.00	681
LYMPHOMA-LYMPHOCYT	CHEM.	0.50	340
LYMPHOMA-LYMPHOCYT	PAT. SUPPORT. CARE	0.50	340
NON-HODGKIN LYMPH.	RAD.	1.00	681
NON-HODGKIN LYMPH.	CHEM.	1.00	681
NON-HODGKIN LYMPH.	PHARM./TOX.	0.50	340
NON-HODGKIN LYMPH.	PAT. SUPPORT. CARE	1.00	681
MYELOMA	CHEM.	2.00	1,362
MYELOMA	PAT. SUPPORT. CARE	2.00	1,362
BLADDER	CHEM.	0.50	340
BLADDER	PAT. SUPPORT. CARE	0.50	340
BREAST	SURG.	3.00	2,042
BREAST	RAD.	3.00	2,042
BREAST	CHEM.	6.00	4,085
BREAST	PHARM./TOX.	1.00	681
BREAST	PAT. SUPPORT. CARE	3.00	2,042
COLORECTAL	SURG.	2.00	1,362
COLORECTAL	RAD.	1.00	681
COLORECTAL	CHEM.	2.00	1,362
COLORECTAL	PAT. SUPPORT. CARE	4.00	2,723
ESOPHAGEAL	CHEM.	0.50	340
ESOPHAGEAL	PAT. SUPPORT. CARE	0.50	340
HEAD AND NECK	SURG.	1.00	681

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
HEAD AND NECK	RAD.	0.50	340
HEAD AND NECK	CHEM.	1.50	1,021
HEAD AND NECK	PAT. SUPPORT. CARE	0.50	340
KIDNEY	CHEM.	1.00	681
KIDNEY	PAT. SUPPORT. CARE	1.00	681
LUNG	SURG.	3.00	2,042
LUNG	RAD.	3.00	2,042
LUNG	CHEM.	5.00	3,404
LUNG	IMM.	3.00	2,042
LUNG	PAT. SUPPORT. CARE	4.00	2,723
MELANOMA	SURG.	0.50	340
MELANOMA	CHEM.	0.50	340
MELANOMA	IMM.	0.50	340
MELANOMA	PAT. SUPPORT. CARE	0.50	340
PANCREATIC	CHEM.	0.50	340
PANCREATIC	PAT. SUPPORT. CARE	0.50	340
PROSTATIC	CHEM.	0.50	340
PROSTATIC	PAT. SUPPORT. CARE	0.50	340
SARCOMAS (GEN.)	CHEM.	0.50	340
SARCOMAS (GEN.)	PAT. SUPPORT. CARE	0.50	340
STOMACH	SURG.	0.50	340
STOMACH	CHEM.	0.50	340
STOMACH	PAT. SUPPORT. CARE	0.50	340
TESTICULAR	SURG.	0.50	340
TESTICULAR	CHEM.	2.00	1,362
TESTICULAR	PAT. SUPPORT. CARE	1.00	681
OTHER CLINICAL TRIALS RESEARCH		* 8.60*	5,854
LEUK.-ACUTE GRAN.	PROTOCOL-REL. STAT	0.30	204
LEUK.-ACUTE LYMPH.	PROTOCOL-REL. STAT	0.20	136
LEUK.-CHRON. GRAN.	PROTOCOL-REL. STAT	0.10	68
LYMPHOMA-HODGKIN'S	PROTOCOL-REL. STAT	0.50	340
NON-HODGKIN LYMPH.	PROTOCOL-REL. STAT	0.50	340
BREAST	PROTOCOL-REL. STAT	1.00	681
COLORECTAL	PROTOCOL-REL. STAT	0.50	340
HEAD AND NECK	PROTOCOL-REL. STAT	0.50	340
LUNG	PROTOCOL-REL. STAT	0.50	340
TESTICULAR	PROTOCOL-REL. STAT	0.20	136
LEUK.-ACUTE GRAN.	DATA PROC. & SUPP.	0.50	340
LYMPHOMA-HODGKIN'S	DATA PROC. & SUPP.	0.50	340
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	340
BREAST	DATA PROC. & SUPP.	1.00	681
COLORECTAL	DATA PROC. & SUPP.	0.50	340
HEAD AND NECK	DATA PROC. & SUPP.	0.50	340
LUNG	DATA PROC. & SUPP.	0.50	340
TESTICULAR	DATA PROC. & SUPP.	0.30	204
NON-HODGKIN LYMPH.	DATA PROC. & SUPP.	0.50	340
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 8.90*	6,059
LEUK.-ACUTE GRAN.	CELL KINETICS	0.20	136
LEUK.-ACUTE LYMPH.	CELL KINETICS	0.20	136
LEUK.-ACUTE LYMPH.	MARKERS	0.30	204
LEUK.-CHRON. GRAN.	CELL KINETICS	0.10	68

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
LEUK.-CHRON. GRAN.	MARKERS	0.10	68
LEUK.-CHRON LYMPH.	CELL KINETICS	0.50	340
LEUK.-CHRON LYMPH.	MARKERS	0.50	340
LYMPHOMA-HODGKIN'S	BLOOD PRODUCTS	0.50	340
NON-HODGKIN LYMPH.	CELL KINETICS	1.00	681
NON-HODGKIN LYMPH.	MARKERS	1.00	681
NON-HODGKIN LYMPH.	BLOOD PRODUCTS	0.50	340
MYELOMA	TRANSFUSION RES.	1.00	681
POLYCYTHERMIA VERA	CELL KINETICS	0.50	340
POLYCYTHERMIA VERA	TRANSFUSION RES.	0.50	340
HEAD AND NECK	NUTRITION	0.50	340
LUNG	SPEC. PHARM./TOX.	0.50	340
LUNG	MARKERS	0.50	340
STOMACH	NUTRITION	0.50	340
NEW YORK UNIVERSITY MEDICAL CENTER	N01CM17473 CTEP		94,700
PROGRAM MANAGEMENT		* 10.00*	9,470
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	28,410
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	56,820
CNS TUMORS	RAD.	30.00	28,410
CNS TUMORS	CHEM.	30.00	28,410
NORTH CAROLINA, UNIVERSITY OF	N01CM17471 CTEP		161,581
PROGRAM MANAGEMEN T		* 10.00*	16,158
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	48,474
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	96,949
CNS TUMORS	RAD.	30.00	48,474
CNS TUMORS	CHEM.	30.00	48,474
NORTHERN CALIFORNIA CANCER PROGRAM	N01CM87154 CTEP		7,251
PHASE IV CLINICAL TRIALS		*100.00*	7,251
HEAD AND NECK	SURG.	30.00	2,175
HEAD AND NECK	RAD.	30.00	2,175
HEAD AND NECK	CHEM.	20.00	1,450
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	1,450
NORTHRUP SERVICES, INC.	N01CM07286 A.P.		65,601
ACQUISITION OF MATERIALS		* 20.00*	13,120
ANIMALS			
BASIC SCREEN		* 55.00*	36,081
ANIMALS			
VERIFICATION SCREEN		* 25.00*	16,400
ANIMALS			

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
NORTHWESTERN UNIVERSITY	N01CM17363		47,897
ACQUISITION OF MATERIALS	A.P.		
ANIMALS		* 20.00*	9,579
BASIC SCREEN		* 55.00*	26,343
ANIMALS			
VERIFICATION SCREEN		* 25.00*	11,974
ANIMALS			
OHIO STATE UNIVERSITY RESEARCH	N01CM67060		15,070
PROGRAM MANAGEMENT	CTEP		
ADMINISTRATION		* 10.00*	1,507
PHASE II CLINICAL TRIALS		* 30.00*	4,521
CNS TUMORS			
PHASE III CLINICAL TRIALS		* 60.00*	9,042
CNS TUMORS		30.00	4,521
CNS TUMORS		30.00	4,521
OHIO STATE UNIVERSITY RESEARCH	N01CM17375		104,409
PHARMACOLOGY/TOXICOLOGY	D.T.P.		
ANAL. METH.		* 25.00*	26,102
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 75.00*	78,307
NON-SPECIFIC			
OHIO STATE UNIVERSITY RESEARCH	N01CM97264		8,400
PHARMACOLOGY/TOXICOLOGY	D.T.P.		
PHARMACOLOGY		* 100.00*	8,400
ONTARIO CANCER INSTITUTE	N01CM07418		239,104
PHASE II CLINICAL TRIALS	CTEP		
LUNG		* 20.00*	47,821
LUNG		10.00	23,910
LUNG		10.00	23,910
PHASE III CLINICAL TRIALS		* 60.00*	143,462
LUNG		30.00	71,731
LUNG		30.00	71,731
PHASE IV CLINICAL TRIALS		* 20.00*	47,821
LUNG		10.00	23,910
LUNG		10.00	23,910
PHARM-ECO	N01CM17487		287,466
PROCUREMENT OF PRECLINICAL MATERIAL	D.T.P.		
SYNTHETICS		* 50.00*	143,733
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	143,733
PRODUCTION, SYNTHETICS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
PHILIPS ROXANE LABORATORIES, INC.	N01CM67053		99,480
FORMULATION	D.T.P.		
DEVEL. OF EXP. FORMULATIONS		* 20.00*	19,896
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	79,584
FORMULATION		70.00	69,636
ANALYTICAL AND QUALITY CONTROL		10.00	9,948
PITTSBURGH, UNIVERSITY OF	N01CB23876B		1,199,870
CTEP			
PHASE III CLINICAL TRIALS		*100.00*	1,199,870
BREAST SURG.		20.00	239,974
BREAST RAD.		20.00	239,974
BREAST CHEM.		20.00	239,974
BREAST IMM.		20.00	239,974
COLORECTAL SURG.		5.00	59,994
COLORECTAL RAD.		5.00	59,994
COLORECTAL CHEM.		5.00	59,994
COLORECTAL IMM.		5.00	59,994
PITTSBURGH, UNIVERSITY OF	N01CM77177		800,282
CTEP			
PHASE IV CLINICAL TRIALS		*100.00*	800,282
COLORECTAL SURG.		40.00	320,113
COLORECTAL RAD.		10.00	80,028
COLORECTAL CHEM.		50.00	400,141
POLYSCIENCES, INC.	N01CM07300		270,096
D.T.P.			
ACQUISITION OF MATERIALS		*100.00*	270,096
NEW AGENT PROCUREMENT, PLANT PRODUCTS		79.00	213,376
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		10.00	27,010
BIOASSAY OF NATURAL PRODUCTS		11.00	29,711
PURDUE RESEARCH FOUNDATION	N01CM97296		183,983
D.T.P.			
ACQUISITION OF MATERIALS		* 85.00*	156,386
NEW AGENT PROCUREMENT, PLANT PRODUCTS		65.00	119,589
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		15.00	27,597
BIOASSAY OF NATURAL PRODUCTS		5.00	9,199
PROCUREMENT OF PRECLINICAL MATERIAL		* 15.00*	27,597
PLANT PRODUCTS		10.00	18,398
FERMENTATION/ANTIBIOTICS		5.00	9,199
RESEARCH TRIANGLE INSTITUTE	N01CM07352		6,705
D.T.P.			
ACQUISITION OF MATERIALS		*100.00*	6,705
NEW AGENT PROCUREMENT, SYNTHETICS			

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
RESEARCH TRIANGLE INSTITUTE	N01CM97313		212,570
PROCUREMENT OF PRECLINICAL MATERIAL	D.T.P.		
RADIOLABELED MATERIALS	* 80.00*	170,056	
PROD. AND FORM. FOR CLINICAL TRIALS	* 20.00*	42,514	
RADIOLABEL			
SAINT LOUIS UNIVERSITY SCHOOL OF	N01CM57020		24,735
PROGRAM MANAGEMENT	CTEP		
ADMINISTRATION	* 10.00*	2,474	
PHASE II CLINICAL TRIALS	* 30.00*	7,421	
CNS TUMORS			
PHASE III CLINICAL TRIALS	* 60.00*	14,841	
CNS TUMORS	30.00	7,421	
CNS TUMORS	30.00	7,421	
SASCO, INC.	N01CM90164		234,000
ACQUISITION OF MATERIALS	A.P.		
ANIMALS	* 20.00*	46,800	
BASIC SCREEN	* 55.00*	128,700	
ANIMALS			
VERIFICATION SCREEN	* 25.00*	58,500	
ANIMALS			
SIDNEY FARBER CANCER INSTITUTE	N01CM97276		100,000
PHASE I CLINICAL TRIALS	CTEP		
NON-SPECIFIC	* 71.00*	71,000	
PHASE II CLINICAL TRIALS			
LEUK.-ACUTE GRAN.	* 29.00*	29,000	
LEUK.-ACUTE LYMPH.	2.00	2,000	
GENERAL	2.00	2,000	
BREAST	5.00	5,000	
COLORRECTAL	5.00	5,000	
LUNG	5.00	5,000	
MELANOMA	5.00	5,000	
SIMONSEN LABORATORIES	N01CM50578		245,700
ACQUISITION OF MATERIALS	A.P.		
ANIMALS	* 20.00*	49,140	
BASIC SCREEN	* 55.00*	135,135	
ANIMALS			
VERIFICATION SCREEN	* 25.00*	61,425	
ANIMALS			

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
SIMONSEN LABORATORIES	N01CM77166 A.P.		810,298
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	162,060
BASIC SCREEN ANIMALS		* 55.00*	445,664
VERIFICATION SCREEN ANIMALS		* 25.00*	202,575
SIMONSEN LABORATORIES	N01CM97247 A.P.		358,090
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	71,618
BASIC SCREEN ANIMALS		* 55.00*	196,950
VERIFICATION SCREEN ANIMALS		* 25.00*	89,523
SISA, INC.	N01CM07354 D.T.P.		16,074
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS		*100.00*	16,074
SMALL BUSINESS ADMINISTRATION	N01CM43719 D.T.P.		144,000
ACQUISITION OF MATERIALS ANIMAL CELLS		* 70.00*	100,800
CELLULAR/SUBCELLULAR STUDIES		* 30.00*	43,200
MOLECULAR BIOLOGY		10.00	14,400
RADIOIMMUNE ASSAY		20.00	28,800
SOUTH FLORIDA, UNIVERSITY OF	N01CM87220 CTEP		64,328
PHASE IV CLINICAL TRIALS		*100.00*	64,328
HEAD AND NECK SURG.		30.00	19,298
HEAD AND NECK RAD.		30.00	19,298
HEAD AND NECK CHEM.		20.00	12,866
HEAD AND NECK PAT. SUPPORT. CARE		20.00	12,866
SOUTHERN ANIMAL FARMS	N01CM50599 A.P.		231,660
ACQUISITION OF MATERIALS ANIMALS		* 20.00*	46,332
BASIC SCREEN ANIMALS		* 55.00*	127,413
VERIFICATION SCREEN ANIMALS		* 25.00*	57,915

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NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
SOUTHERN ANIMAL FARMS	N01CM97245		164,640
ACQUISITION OF MATERIALS	A.P.		
ANIMALS		* 20.00*	32,928
BASIC SCREEN		* 55.00*	90,552
ANIMALS			
VERIFICATION SCREEN		* 25.00*	41,160
ANIMALS			
SOUTHERN RESEARCH INSTITUTE	N01CM07260		25,100
ACQUISITION OF MATERIALS	D.T.P.		
NEW AGENT PROCUREMENT, SYNTHETICS		* 100.00*	25,100
SOUTHERN RESEARCH INSTITUTE	N01CM87162		187,950
PHARMACOLOGY/TOXICOLOGY	D.T.P.		
PHARMACOLOGY		* 100.00*	187,950
ANAL. METH.		80.00	150,360
		20.00	37,590
SOUTHERN RESEARCH INSTITUTE	N01CM97263		235,000
PHARMACOLOGY/TOXICOLOGY	D.T.P.		
SPECIAL TOXICITY STUDIES		* 70.00*	164,500
TREATMENT STUDIES		* 30.00*	70,500
CHEMOTHERAPY			
SOUTHERN RESEARCH INSTITUTE	N01CM97309		2,008,237
ACQUISITION OF MATERIALS	D.T.P.		
BIOASSAY OF NATURAL PRODUCTS		* 1.00*	20,082
BASIC SCREEN		* 32.00*	642,636
PRIMARY SCREENING, IN VITRO		4.00	80,329
PRIMARY SCREENING, IN VIVO		16.00	321,318
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		10.00	200,824
ANALOG SCREENING		2.00	40,165
VERIFICATION SCREEN		* 26.00*	522,142
DETAILED DRUG EVALUATION, IN VIVO		14.00	281,153
DET. DRUG EVAL., RELATED NEW MODEL DEVEL		10.00	200,824
ANTIVIRAL ACTIVITY		2.00	40,165
PHARMACOLOGY/TOXICOLOGY		* 2.00*	40,165
SPECIAL TOXICITY STUDIES		1.00	20,082
BIOASSAY-DRUG METAB.		1.00	20,082
CELLULAR/SUBCELLULAR STUDIES		* 1.00*	20,082
CELL KINETICS		1.00	20,082
TREATMENT STUDIES		* 38.00*	763,130
COMB. MODAL. THERAPY		38.00	763,130
COMB. MODAL. THERAPY - SURG.		17.00	341,400
COMB. MODAL. THERAPY - CHEM.		21.00	421,730

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
STANFORD RESEARCH INSTITUTE	N01CM87207B		60,160
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS	CTEP	*100.00*	60,160
STANFORD RESEARCH INSTITUTE	N01CM07351		14,919
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS	D.T.P.	*100.00*	14,919
STANFORD RESEARCH INSTITUTE	N01CM87183		552,544
PROCUREMENT OF PRECLINICAL MATERIAL ANALYTICAL AND QUALITY CONTROL	D.T.P.	* 20.00*	110,509
PROD. AND FORM. FOR CLINICAL TRIALS ANALYTICAL AND QUALITY CONTROL		* 80.00*	442,035
STANFORD RESEARCH INSTITUTE	N01CM97256		247,570
PROCUREMENT OF PRECLINICAL MATERIAL RADIOLABELED MATERIALS	D.T.P.	* 80.00*	198,056
PROD. AND FORM. FOR CLINICAL TRIALS RADIOLABEL		* 20.00*	49,514
STARKS ASSOCIATES, INC.	N01CM07357		24,334
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS	D.T.P.	*100.00*	24,334
STARKS ASSOCIATES, INC.	N01CM17374		575,789
PROCUREMENT OF PRECLINICAL MATERIAL SYNTHETICS	D.T.P.	* 50.00*	287,895
PROD. AND FORM. FOR CLINICAL TRIALS PRODUCTION, SYNTHETICS		* 50.00*	287,895
STARKS ASSOCIATES, INC.	N01CM87206		555,000
ACQUISITION OF MATERIALS NEW AGENT PROCUREMENT, SYNTHETICS	D.T.P.	*100.00*	555,000
DATA PROCESSING AND SUPPORT		70.00	388,500
		30.00	166,500
TACONIC FARMS	N01CM50597		25,500
ACQUISITION OF MATERIALS ANIMALS	A.P.	* 20.00*	5,100
BASIC SCREEN ANIMALS		* 55.00*	14,025
VERIFICATION SCREEN ANIMALS		* 25.00*	6,375

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
TENNESSEE UNIVERSITY CENTER FOR HEALTH	N01CM17472		145,498
CTEP			
PROGRAM MANAGEMENT		* 10.00*	14,550
ADMINISTRATION			
PHASE II CLINICAL TRIALS		* 30.00*	43,649
CNS TUMORS	CHEM.		
PHASE III CLINICAL TRIALS		* 60.00*	87,299
CNS TUMORS	RAD.	30.00	43,649
CNS TUMORS	CHEM.	30.00	43,649
TEXAS, UNIVERSITY OF, MEDICAL BRANCH	N01CM87221		40,250
CTEP			
PHASE IV CLINICAL TRIALS		*100.00*	40,250
HEAD AND NECK	SURG.	30.00	12,075
HEAD AND NECK	RAD.	30.00	12,075
HEAD AND NECK	CHEM.	20.00	8,050
HEAD AND NECK	PAT. SUPPORT. CARE	20.00	8,050
TEXAS, UNIVERSITY OF, SYSTEM CANCER	N01CM07406		277,707
CTEP			
PHASE II CLINICAL TRIALS		* 55.00*	152,739
BREAST	CHEM.	15.00	41,656
COLORECTAL	CHEM.	15.00	41,656
HEAD AND NECK	CHEM.	5.00	13,885
LUNG	CHEM.	5.00	13,885
MELANOMA	CHEM.	10.00	27,771
SARCOMAS (GEN.)	CHEM.	5.00	13,885
PHASE III CLINICAL TRIALS		* 45.00*	124,968
BREAST	CHEM.	10.00	27,771
COLORECTAL	CHEM.	5.00	13,885
HEAD AND NECK	CHEM.	5.00	13,885
LUNG	CHEM.	5.00	13,885
MELANOMA	CHEM.	15.00	41,656
SARCOMAS (GEN.)	CHEM.	5.00	13,885
TEXAS, UNIVERSITY OF, SYSTEM CANCER	N01CM97277		253,000
CTEP			
PHASE I CLINICAL TRIALS		* 71.00*	179,630
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS		* 29.00*	73,370
LEUK.-ACUTE GRAN.	CHEM.	2.00	5,060
LEUK.-ACUTE LYMPH.	CHEM.	2.00	5,060
GENERAL	CHEM.	5.00	12,650
BREAST	CHEM.	5.00	12,650
COLORECTAL	CHEM.	5.00	12,650
LUNG	CHEM.	5.00	12,650
MELANOMA	CHEM.	5.00	12,650

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
TEXAS, UNIVERSITY OF, SYSTEM CANCER	N01CM87185		71,250
PHARMACOLOGY/TOXICOLOGY	D.T.P.	* 60.00*	42,750
PHARMACOLOGY			
PHASE I CLINICAL TRIALS		* 40.00*	28,500
NON-SPECIFIC	PHARM./TOX.		
TEXAS, UNIVERSITY OF	N01CM07417		97,970
	CTEP		
PHASE II CLINICAL TRIALS		* 20.00*	19,594
LUNG	RAD.	10.00	9,797
LUNG	CHEM.	10.00	9,797
PHASE III CLINICAL TRIALS		* 60.00*	58,782
LUNG	RAD.	30.00	29,391
LUNG	CHEM.	30.00	29,391
PHASE IV CLINICAL TRIALS		* 20.00*	19,594
LUNG	RAD.	10.00	9,797
LUNG	CHEM.	10.00	9,797
UPJOHN COMPANY	N01CM07320		803,205
	D.T.P.		
ACQUISITION OF MATERIALS		* 85.00*	682,724
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	666,660
DATA PROCESSING AND SUPPORT		2.00	16,064
BASIC SCREEN		* 15.00*	120,481
PRIMARY SCREENING, IN VITRO		10.00	80,321
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	40,160
VERMONT, UNIVERSITY OF, COLLEGE OF	N01CM97278		122,316
	CTEP		
PHASE I CLINICAL TRIALS		* 71.00*	86,844
NON-SPECIFIC	CHEM.		
PHASE II CLINICAL TRIALS		* 29.00*	35,472
LEUK.-ACUTE GRAN.	CHEM.	2.00	2,446
LEUK.-ACUTE LYMPH.	CHEM.	2.00	2,446
GENERAL	CHEM.	5.00	6,116
BREAST	CHEM.	5.00	6,116
COLORECTAL	CHEM.	5.00	6,116
LUNG	CHEM.	5.00	6,116
MELANOMA	CHEM.	5.00	6,116
VETERANS ADMINISTRATION	Y01CM70107		500,000
	CTEP		
PHASE III CLINICAL TRIALS		* 40.00*	200,000
HEAD AND NECK	CHEM.	5.00	25,000
HEAD AND NECK	IMM.	5.00	25,000
LUNG	RAD.	10.00	50,000
LUNG	CHEM.	20.00	100,000
PHASE IV CLINICAL TRIALS		* 40.00*	200,000
COLORECTAL	SURG.	5.00	25,000

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
COLORECTAL	RAD.	5.00	25,000
COLORECTAL	CHEM.	5.00	25,000
ESOPHAGEAL	RAD.	2.50	12,500
ESOPHAGEAL	CHEM.	2.50	12,500
LUNG	SURG.	5.00	25,000
LUNG	CHEM.	5.00	25,000
LUNG	IMM.	5.00	25,000
PANCREATIC	CHEM.	2.50	12,500
STOMACH	CHEM.	2.50	12,500
OTHER CLINICAL TRIALS RESEARCH		* 20.00*	100,000
COLORECTAL	DATA PROC. & SUPP.	2.50	12,500
ESOPHAGEAL	DATA PROC. & SUPP.	2.50	12,500
HEAD AND NECK	DATA PROC. & SUPP.	2.50	12,500
LUNG	DATA PROC. & SUPP.	5.00	25,000
PANCREATIC	DATA PROC. & SUPP.	2.50	12,500
STOMACH	DATA PROC. & SUPP.	5.00	25,000
VSE, CORPORATION	N01CM07251 D.T.P.		1,032,314
ACQUISITION OF MATERIALS		* 14.00*	144,524
BIOASSAY OF NATURAL PRODUCTS			
BASIC SCREEN		* 58.00*	598,742
PRIMARY SCREENING, IN VITRO		5.00	51,616
PRIMARY SCREENING, IN VIVO		53.00	547,126
VERIFICATION SCREEN		* 20.00*	206,463
DETAILED DRUG EVALUATION, IN VIVO			
TREATMENT STUDIES		* 8.00*	82,585
CHEMOTHERAPY			
WARNER LAMPERT	N01CM07292 BRMP		37,190
ACQUISITION OF MATERIALS		* 100.00*	37,190
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		30.00	11,157
ANIMALS		70.00	26,033
WARNER LAMPERT	N01CM17491 D.T.P.		329,611
PROCUREMENT OF PRECLINICAL MATERIAL		* 50.00*	164,806
SYNTHETICS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 50.00*	164,806
PRODUCTION, SYNTHETICS			
WARNER LAMPERT	N01CM07379 D.T.P.		687,000
ACQUISITION OF MATERIALS		* 85.00*	583,950
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		83.00	570,210
DATA PROCESSING AND SUPPORT		2.00	13,740
BASIC SCREEN		* 15.00*	103,050
PRIMARY SCREENING, IN VITRO		10.00	68,700
PRIMARY SCREEN., RELATED NEW MODEL DEVEL		5.00	34,350

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
WASHINGTON, UNIVERSITY OF	N01CM97282		279,760
OTHER CLINICAL TRIALS RESEARCH	CTEP		
NON-SPECIFIC		*100.00*	279,760
WAYNE STATE UNIVERSITY	N01CM07404		224,593
PHASE II CLINICAL TRIALS	CTEP		
BREAST	CHEM.	* 55.00*	123,526
COLORRECTAL	CHEM.	15.00	33,689
HEAD AND NECK	CHEM.	15.00	33,689
LUNG	CHEM.	5.00	11,230
MELANOMA	CHEM.	5.00	11,230
SARCOMAS (GEN.)	CHEM.	10.00	22,459
PHASE III CLINICAL TRIALS		5.00	11,230
BREAST	CHEM.	* 45.00*	101,067
COLORRECTAL	CHEM.	10.00	22,459
HEAD AND NECK	CHEM.	5.00	11,230
LUNG	CHEM.	5.00	11,230
MELANOMA	CHEM.	5.00	11,230
SARCOMAS (GEN.)	CHEM.	15.00	33,689
		5.00	11,230
WAYNE STATE UNIVERSITY	N01CM07408		96,915
PHASE IV CLINICAL TRIALS	CTEP		
STOMACH	SURG.	* 90.00*	87,224
STOMACH	RAD.	25.00	24,229
STOMACH	CHEM.	25.00	24,229
CLINICAL TRIALS SUPPORTIVE RESEARCH		40.00	38,766
STOMACH	MARKERS	* 10.00*	9,692
WAYNE STATE UNIVERSITY	N01CM97279		127,636
PHASE I CLINICAL TRIALS	CTEP		
NON-SPECIFIC	CHEM.	* 71.00*	90,622
PHASE II CLINICAL TRIALS		* 29.00*	37,014
LEUK.-ACUTE GRAN.	CHEM.	2.00	2,553
LEUK.-ACUTE LYMPH.	CHEM.	2.00	2,553
GENERAL	CHEM.	5.00	6,382
BREAST	CHEM.	5.00	6,382
COLORRECTAL	CHEM.	5.00	6,382
LUNG	CHEM.	5.00	6,382
MELANOMA	CHEM.	5.00	6,382
WELLCOME FOUNDATION, LTD.	N01CM17489		1,649,277
ACQUISITION OF MATERIALS	BRMP		
NEW AGENT PROCUREMENT, FERMENT/ANTIBIOT		*100.00*	1,649,277
ANIMALS		30.00	494,783
		70.00	1,154,494

BEST DOCUMENT AVAILABLE

NAME	# & AREA	PERCENT OF EFFORT	DOLLAR LEVEL
WISCONSIN, UNIVERSITY OF	N01CM97280		99,192
CTEP			
PHASE I CLINICAL TRIALS		* 71.00*	70,426
NON-SPECIFIC CHEM.			
PHASE II CLINICAL TRIALS		* 29.00*	28,766
LEUK.-ACUTE GRAN. CHEM.		2.00	1,984
LEUK.-ACUTE LYMPH. CHEM.		2.00	1,984
GENERAL CHEM.		5.00	4,960
BREAST CHEM.		5.00	4,960
COLORECTAL CHEM.		5.00	4,960
LUNG CHEM.		5.00	4,960
MELANOMA CHEM.		5.00	4,960
YALE UNIVERSITY SCHOOL OF MEDICINE	N01CM07339		186,643
CTEP			
PHASE IV CLINICAL TRIALS		* 90.00*	167,979
STOMACH SURG.		25.00	46,661
STOMACH RAD.		25.00	46,661
STOMACH CHEM.		40.00	74,657
CLINICAL TRIALS SUPPORTIVE RESEARCH		* 10.00*	18,664
STOMACH MARKERS			
YAMANOUCHI PHARMACEUTICAL CO.	N01CM97307		210,000
D.T.P.			
FORMULATION		* 20.00*	42,000
DEVEL. OF EXP. FORMULATIONS			
PROD. AND FORM. FOR CLINICAL TRIALS		* 80.00*	168,000
FORMULATION		70.00	147,000
ANALYTICAL AND QUALITY CONTROL		10.00	21,000

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TABLE IV
DESCRIPTION OF CONTRACTS
IN THE
DIVISION OF CANCER TREATMENT

AGRICULTURE, DEPARTMENT OF (TRANSFER OF FUNDS) (Y01-CM4-0001)

Under the Transfer of Funds Agreement with the Economic Botany Laboratory of the U.S.D.A., worldwide general plant collections and recollections are made. The general plant collections (approximately 1,000 plants) are shipped to Raltech Scientific Services, Inc. for extraction and the extracts are screened in vitro and in vivo for antineoplastic activity. Confirmed active plants are recollected in large quantities (100 to 300 lbs.) on a priority based on the needs of the DCT for shipment to the fractionating chemists. The nomenclature of all plants acquisitioned is being continuously reviewed and corrected, and a computer file of all plants is being maintained.

ALABAMA, UNIVERSITY OF (N01-CM0-7355)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

ALBANY MEDICAL CENTER (N01-CB5-3940)

This contract was recently transferred from DCBD to the Biological Evaluation Branch, CTEP. It consists of a clinical trial designed to evaluate intrapleural BCG with or without cutaneous BCG in patients with lung cancer. The study is close to completion. Final data are expected within one or two years.

ALBANY MEDICAL COLLEGE (N01-CM5-7032)

This contract is designed to support prospective, randomized controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. In addition, the contractor has completed a pilot study within the G.I. Tumor Study Group in advanced colon cancer.

ALBERT EINSTEIN SCHOOL OF MEDICINE (N01-CM1-7340)

This contract will support a Phase I evaluation of topical retinoids, vitamin A derivatives, to be applied directly onto the surface of the cervical mucosa. After a careful evaluation of toxicities, a Phase III trial will be initiated, which shall attempt to identify the role of vitamin A analogs as chemopreventive agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial shall try to demonstrate that cellular differentiations and maturation can be induced by vitamin A analogs.

ALDRICH CHEMICAL COMPANY, INC. (N01-CM1-7492)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

AMERICAN COLLEGE OF RADIOLOGY (N01-CM8-7219)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

AMERICAN TYPE CULTURE COLLECTION (N01-CM0-5725)

This contract supplies the Government with substantial quantities of well-characterized normal and neoplastic mammalian tissue culture cells and receives, processes, distributes, stores and maintains fresh human leukemic cells and tissues. Special cell cultures are grown under specified conditions and all harvested cells are required to be metabolically active and delivered to the Government within one hour. Complete records are maintained on all biological materials handled under the contract.

ARIZONA, UNIVERSITY OF (N01-CM1-7497)

This is one of four new contracts devoted to the development of a human tumor clonogenic assay for drug screening. As the first phase of the project, the contractor will be engaged in a pilot study which involves testing 50 compounds blind against a spectrum of human tumors. Upon the completion of testing, the data will be subjected to statistical analysis. The contractor also is conducting limited developmental studies such as comparisons of enzymatic and mechanical tissue disaggregation techniques and comparisons of different media. The objective of these latter studies is to explore ways of improving cloning efficiency and cost efficiency of the system for mass screening.

ARIZONA, UNIVERSITY OF (N01-CM1-7500)

This contract will support a Phase I evaluation of topical retinoids, vitamin A derivatives, to be applied directly onto the surface of the cervical mucosa. After a careful evaluation of toxicities, a Phase III trial will be initiated, which shall attempt to identify the role of vitamin A analogs as chemopreventive agents. Women with abnormal pap smear cytology will be prospectively analyzed in a double blind trial to see if retinoids can improve dysplastic cervical morphology. This chemopreventive trial shall try to demonstrate that cellular differentiation and maturation can be induced by vitamin A analogs.

ARIZONA STATE UNIVERSITY (N01-CM9-7262)

This contract provides for the preparation of extracts and isolation and identification of antineoplastic agents from marine organisms. A large number of active extracts has been produced and 20-35 of the most active organisms have been recollected in large amounts and are undergoing fractionation studies. The marine organisms are purchased by NCI and are obtained by outside zoologists. This project is being terminated due to budgetary restrictions. Presently, eight good leads are undergoing chemical isolation of the active antineoplastic compound(s).

ARIZONA STATE UNIVERSITY (N01-CM9-7297)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the U.S. Department of Agriculture.

ARTHUR D. LITTLE, INC. (N01-CM8-7163)

This contract conducts an investigative program of the pharmacological properties of experimental antitumor agents. Analytical methods are developed to measure the drugs in body tissues and fluids and are then used to determine pharmacological parameters such as plasma level decay curves, routes of excretion, bioavailability, etc. When drug biotransformation is observed, the metabolites are identified wherever possible.

ARTHUR D. LITTLE, INC. (N01-CM8-7186)

The objective of this contract is to obtain basic information on the cytotoxic, biochemical and therapeutic effects of new antitumor agents that will aid DCT in deciding whether to develop drugs to clinical trial and in assigning priorities to those drugs that are under development. Experiments are conducted to: (1) provide clear leads as to the biologically significant properties of chemically unique new drugs; (2) determine if antitumor agents with novel structures have biochemical activities similar to those of clinically evaluated drugs; and (3) determine if structural analogs being considered for development are also biochemical analogs of their parent compound.

ARTHUR D. LITTLE, INC. (N01-CM9-7288)

This contract provides cell culture assays as an aid in the isolation and purification of potential antitumor agents of plant and animal origin. Occasional synthetic materials are also tested in these in vitro assays. During this year, a significant effort was also put into the development of new in vitro screening techniques.

ARTHUR D. LITTLE, INC. (N01-CM0-7257)

The capability for evaluating chemical compounds for radiation sensitizing properties is provided by this resource. Various physico-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radiosensitizing characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiosensitization using mammalian cell cultures. Compounds which appear to be superior to the standard - misonidazole - will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radiosensitizers), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All testing will be compared with the standard - misonidazole. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds. This contract should provide new radiosensitizers or leads in developing new types (classes) of radiosensitizing compounds.

ARTHUR D. LITTLE, INC. (N01-CM0-7302) (FORMERLY N01-CM5-3765)

This contract utilizes a variety of leukemia and solid animal tumor models in carrying out its three major objectives: (1) evaluation of analogs of known antitumor drugs and new structural classes; (2) combination chemotherapy studies; and (3) combined modality studies involving drugs plus radiation. To assess potential superiority of analogs, direct comparison tests of analogs and a designated parent are carried out in systems designed for each compound class. The results of these structure-activity tests are reported rapidly to NCI and to the suppliers to guide future synthetic efforts. Combinations of drugs are

ARTHUR D. LITTLE, INC. (N01-CM0-7302) (CONTINUED)

examined in a variety of tumor systems to determine their potential superiority over the drugs used alone. The effects of radiation and combinations of radiation with chemotherapeutic agents, radiosensitizers, and radioprotectors also are examined in the treatment of leukemic and solid tumors.

ARTHUR D. LITTLE, INC. (N01-CM0-7331)

The major objective of this contract is to develop three in vitro test systems for mass screening of natural products. When these in vitro screens are optimized and evaluated against 50 known pure natural products and 1000 unknown crude extracts, they will then be put either in the Natural Products Branch or Drug Evaluation Branch contract laboratories.

ARTHUR D. LITTLE, INC. (N01-CM0-7346)

The current level of testing under this contract is approximately 25,000 L1210-equivalent tests per year. The contract provides for in vivo testing in the P388 leukemia pre-screen, for evaluation of materials in specified tumor panel models, for detailed evaluations requested by members of the NCI staff, and for characterizations and evaluations of tumor models as directed by the NCI Project Officer. All testing is carried out in mice in accordance with the protocols of the NCI Developmental Therapeutics Program (DTP). Materials tested in the P388 leukemia pre-screen are new synthetic compounds and natural products provided by the NCI. Models of the conventional tumor panel now in use under this contract include the B16 melanocarcinoma, colon 38 carcinoma, and L1210 leukemia. Materials tested in the tumor panel models under this contract are selected for the screen by the DTP and consists particularly of materials that have demonstrated activity in the P388 prescreen. Detailed testing usually involves materials of potential clinical interest and includes schedule dependency studies, drug route studies, batch comparisons, and formulation comparisons.

ASH STEVENS, INC. (N01-CM1-7488)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available from the original supplier or commercial sources in the amounts required. About 60% of the effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

BANNER GELATIN PRODUCTS CORPORATION (N01-CM1-7402)

The objectives of this contract are to provide facilities and capabilities for the development and production of soft gelatin capsules containing investigational anticancer agents. The contractor is responsible for conformity to FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. In addition, the contractor is responsible for

BANNER GELATIN PRODUCTS CORPORATION (N01-CM1-7402) (CONTINUED)

stability surveillance on the dosage forms prepared. All products are packaged, labeled and shipped to the NCI for subsequent redistribution to clinical investigators.

BATTELLE MEMORIAL INSTITUTE (N01-CM1-7365)

This service type contract was recompeted in 1980 and a Prime Contract was awarded to Battelle Memorial Laboratories in November 1980 for supervision of subcontractors carrying out the toxicologic evaluation of potential oncolytic agents, biologic response modifiers and other modalities. Through the Prime Contract mechanism, preclinical toxicologic studies of agents under consideration for potential clinical use are handled under a single management-type contract. The work scope is comprised of four tasks as follows: Task I - full protocol studies; Task II - high priority toxicity studies (i.e., any portion of the Protocol of the Toxicology Branch); Task III - specific organ testing; and Task IV - automation of toxicity data, anomaly detection, scheduling of studies, and financial management.

BATTELLE MEMORIAL INSTITUTE (N01-CM6-7099)

This contract is for the testing of materials in human xenograft tumor models using athymic (nude) mice. Currently, materials are tested with the tumor implanted in the subrenal capsule. Presently, one human lung, colon, and mammary xenograft models are employed. Approximately 300 compounds are tested per year in each of the three systems.

BATTELLE MEMORIAL INSTITUTE (N01-CM0-7266)

Battelle Columbus Laboratories conduct in vivo testing of new synthetic materials and natural products of plant, animal, and fermentation origin. In addition, tumor panel testing is conducted upon active materials of interest to the Developmental Therapeutics Program. This testing is conducted at a level of effort of approximately 50,000 L1210 equivalents per year. New synthetic and natural product materials are tested in the P388 pre-screen. Detailed studies, such as schedule dependency studies or experimental form vs bulk drug comparison testing, are conducted on materials in development toward clinical trial when requested by the Project Officer.

BEN VENUE LABORATORIES, INC. (N01-CM9-7298)

This resource contract provides for the development and production of parenteral clinical dosage forms of antitumor agents. The contractor has the capacity for preparing large-scale production batches of dry filled, liquid filled and lyophilized sterile products. Specifically, the contractor performs the following services: (1) formulation development of sterile parenteral products; (2) production of sterile products; (3) quality assurance testing of finished products; and (4) stability surveillance of all dosage forms produced. All products are packaged, labeled and shipped to the NCI for subsequent redistribution to clinical investigators.

BOWMAN GRAY SCHOOL OF MEDICINE (N01-CM6-7054)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is being phased out during this fiscal year.

BRISTOL LABORATORIES (N01-CM3-3751)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, BCNU. The contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the FDA. The NDA has been approved, and the drug is currently on the market. This contract will continue for five years from the NDA approval.

BRISTOL LABORATORIES, INC. (N01-CM6-7051)

This is a no-cost contract concerned with the marketing of the clinically useful nitrosourea, CCNU. The contractor summarized the clinical and other data, and prepared and filed the New Drug Application (NDA) with the FDA. The NDA has been approved, and the drug is currently on the market. This contract will continue for five years from the NDA approval.

BRISTOL LABORATORIES, INC. (N01-CM8-7180)

The primary objective of this contract is the synthesis of analogs of natural products with the expectation of increasing activity while decreasing adverse side effects. The compounds for synthesis are selected in consultation with the Natural Products Branch (DTP) and Analog Development Committee. Two examples of current interest are isoxazoline antibiotics and anthramycins.

BRISTOL LABORATORIES, INC. (N01-CM0-7299) (FORMERLY N01-CM7-7147)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various unique microbes isolated in Bristol's Japanese facility, Bristol Banyu; (2) the use of 10 different in vitro pre-screens to evaluate the fermentations; (3) development of an in vitro assay to assist in quickly isolating the active anticancer agents; (4) dereplication of the materials to determine novelty; (5) chemical isolation of the active component; and (6) production of large quantities of new agents to thoroughly evaluate them in DCT screens.

BRISTOL LABORATORIES, INC. (N01-CM0-7324) (FORMERLY N01-CM7-7138)

The major objective of this fermentation contract is the preparation of novel antibiotics. These new antibiotics are obtained using the fermentation techniques of biotransformation and co-metabolism of antibiotics, chemicals and plant materials. This effort is to convert materials which have solubility, toxicity or marginal activity problems into worthwhile antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain new antineoplastic agents. Each compound is exposed to a selected number of microbes to see if any worthwhile conversions occur.

BRITISH COLUMBIA, UNIVERSITY OF (N01-CM8-7236)

This contract is to evaluate the ability of plant cells to grow and to produce the antineoplastic agent in submerged fermentations. Often, plants which produce antineoplastic agents are found in foreign countries. In order to insure supply of these plants, one must be able to cultivate them or find worldwide sources. Weather and political conditions often make it prohibitive to obtain plant material in sufficient quantity to isolate adequate amounts of the antineoplastic agent for NCI evaluation. The contract is evaluating the possibility of propagating plant cells in submerged culture to produce antineoplastic agents of interest. This would insure local supply of such agents and allow NCI to move quickly to obtain these materials. Eight plants of interest to NCI have been assigned and one has already successfully been propagated to produce the antineoplastic agent of interest. This contract will no be renewed due to budgetary restrictions.

CALIFORNIA, UNIVERSITY OF (N01-CB1-5525)

The purpose of this study is to determine whether BCG therapy alone or BCG combined with tumor cell vaccine will decrease the recurrence rate or prolong survival in melanoma patients with metastases of the regional L-nodes. To date, BCG appears to have some positive effect. This study is planned for continuance.

CALIFORNIA, UNIVERSITY OF (N01-CB8-4250)

The objective of this contract is to produce xenogeneic monospecific antibodies to human lymphoma and leukemia associated antigens. The approach will be to fuse antibody-producing cells of mouse origin with mouse myeloma cells in order to produce hybrid cell lines capable of continuous production of monospecific antibodies against tumor associated antigens in vitro. Two different hybridization techniques will be compared. The specificity of the presumed monoclonal antibodies and the pattern of antigenic tumor-specific determinants on malignant lymphoid cells will be evaluated.

CALIFORNIA, UNIVERSITY OF (N01-CB0-4344)

Adjuvant chemoimmunotherapy is being compared with adjuvant chemotherapy alone in preventing disease recurrence in patients with highly malignant skeletal or soft tissue sarcomas. Currently, there is a trend in favor of the bimodal adjuvant approach. This study will continue to 1983.

CALIFORNIA, UNIVERSITY OF (N01-CM6-7097)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract is currently in phase out status.

CALIFORNIA, UNIVERSITY OF (N01-CM9-7239)

This contract was designed to provide HL-A typing analyses on patients and their families as well as typing of selected donors from the Clinical Center Blood Bank and all platelet donors from the Central Blood Service of Baltimore. Typing data on over 15,000 NIH donors and recipients are on computer file. All typings are performed and entered into the computerized file on the same day that the blood was received. Thus, HL-A typing results for persons bled in Bethesda are consistently available through the computer terminal by the end of the next day. An average of 600 typings are performed annually. This contract will be administered by the Clinical Center beginning in Fiscal Year 1982.

CALIFORNIA, UNIVERSITY OF (N01-CM9-7315)

This contract provides a cyclotron neutron generator, a clinical facility in which to house the equipment, and personnel to support a clinical neutron therapy research program at UCLA. The subcontracts for the cyclotron and for the A/E services were executed in September, 1980. The facility is scheduled for completion in late 1982 and should be operational in early 1983.

CALIFORNIA, UNIVERSITY OF (N01-CM9-7318)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of the lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7349)

This is a Phase I/II clinical trial utilizing human lymphoblastoid interferon that has been recently activated. It is estimated that the Phase I and II portions of the task will be completed in less than two years.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7416)

A member of the Lung Cancer Study Group (LCSG): The LCSG is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a natural history registry, with new surgical and chemopreventive trials to start patient accrual in 7/81. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of postoperative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. New protocols proposed for activation by the group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of the LCSG have been accepted in four major journals.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7420)

This is one of four new contracts devoted to the development of a human tumor clonogenic assay for drug screening. As the first phase of the project, the contractor will be engaged in a pilot study which involves testing 50 compounds blind against a spectrum of human tumors. Upon the completion of testing, the data will be subjected to statistical analysis. The contractor also is conducting limited developmental studies, such as comparisons of enzymatic and mechanical tissue disaggregation techniques and comparisons of different media. The objective of these latter studies is to explore ways of improving cloning efficiency and cost efficiency of the system for mass screening.

CALIFORNIA, UNIVERSITY OF (N01-CM0-7444)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

CANCER THERAPY AND RESEARCH FOUNDATION OF SOUTH TEXAS (N01-CM0-7327)

This is one of four new contracts devoted to the development of a human tumor clonogenic assay for drug screening. As the first phase of the project, the contractor will be engaged in a pilot study which involves testing 50 compounds blind against a spectrum of human tumors. Upon the completion of testing, the data will be subjected to statistical analysis. The contractor also is conducting limited developmental studies, such as comparisons of enzymatic and mechanical tissue disaggregation techniques and comparisons of different media. The objective of these latter studies is to explore ways of improving cloning efficiency and cost efficiency of the system for mass screening.

CARNEGIE MELLON INSTITUTE OF RESEARCH (N01-CM8-7173)

This contractor has conducted an investigation of the relationship of federal regulations to the conduct of clinical treatment research in cancer. The analysis of pertinent FDA and PHS regulations was completed in 1980, the drafting of the final report which speaks to six key questions is being done at present. The questions to be addressed by this analysis will be: (1) whether the research subject is protected from undue risk and coercion by the current federal regulations; (2) the unique status of cancer patients as they relate to FDA and PHS guidelines concerning the risk: benefit ratio spectrum of toxicities of a new therapy; (3) the adequacy and compliance of obtaining informed consent from cancer patients; (4) the ability of IRB's to competently analyze cancer treatment protocols; (5) the way in which the population of cancer patients, particularly those with advanced disease per se their involvement in treatment research; and (6) suggestions for modifying the existing regulations and procedures in order to improve cancer treatment research in the areas listed. This contract terminated in January 1981.

CDP ASSOCIATES, INC. (N01-CM9-7143)

This service contract provides necessary planning, analytical documentation and conference support to the Office of the Director, DCT.

CHARLES RIVER BREEDING LABORATORIES (N01-CM1-7498)

This procurement contract is designed to furnish 156,000 six-week old first generation hybrid mice for Developmental Therapeutics Program contract studies. Fixed-price contract # 263-81-C-0094.

CHARLES RIVER BREEDING LABORATORIES (N01-CM5-0598)

This procurement contract is designed to furnish 234,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for Developmental Therapeutics Program contract studies. The breeding animals originate from the genetic center at this site. Fixed-price contract # 263-81-C-0098.

CHARLES RIVER BREEDING LABORATORIES (N01-CM7-7141)

This primary genetic center has as its objective the development of associated foundation colonies of inbred rodents required for program studies. Pedigreed animals are derived via hysterotomy and foster-suckled in germ-free isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of organisms and are developed as pedigreed expansion colonies in a variety of isolators. Offspring from this second stage are issued to secondary genetic centers which, in turn, produce breeding animals for large-scale production colonies. Classic methods for the maintenance of the animals are followed with respect to environmental controls and microbiological monitoring. A large-scale production colony is maintained in order to provide suitable numbers of rodents for laboratory investigators.

CHARLES RIVER BREEDING LABORATORIES (N01-CM8-7212)

This contract provides for the continual monitoring of the 350 associated isolators within the DCT animal program to determine the flora status of these foundation isolators and to check for specified isolator contaminants. In addition, this contract will provide the correct organisms for establishing flora in new isolators. This contract will expire August 31, 1981. This effort is being recompeted.

CHARLES RIVER BREEDING LABORATORIES (N01-CM9-0163)

This procurement contract provides for the supply of 234,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for the Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers. Fixed-price contract # 263-81-C-0097.

CHARLES RIVER BREEDING LABORATORIES (N01-CM9-7229)

This rodent production center contract supports a production effort designed to furnish animals as required by laboratory programs. Breeding animals are furnished by the government from primary genetic centers.

CHEMICAL ABSTRACTS SERVICE (N01-CM4-3722)

Chemical Abstracts Service (CAS) manages the records of all compounds accessed by the Drug Synthesis & Chemistry Branch, DTP. Their computer system currently holds data on about 340,000 compounds; the data base is growing at the rate of approximately 13,000 compounds annually. Chemical structures and some 150 data elements can be recorded for each compound. The latter include quantity-on-hand, confidentiality, physical properties, origin of compounds, etc. CAS performs data input, quality control, and operates the computer system. The system generates about 40 reports, which distribute new data, monitor the system, and facilitate the management of its information. In addition, users can query the system on-line, for specific compounds, for classes of compounds specified by substructure, or for various data elements. Current objectives are to interlink the chemical and biological systems (operated by different contractors), to improve the compound selection process (by using computer models) and, in general, to improve the efficiency, versatility and convenience of the system.

CHICAGO, UNIVERSITY OF (N01-CM0-7411)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

CHILDREN'S HOSPITAL FOUNDATION, OHIO STATE UNIVERSITY (N01-CM0-7464)

This contract supports a Phase I study of the agent indicine-n-oxide in pediatric patients. This study is expected to enroll 25-30 patients at various doses to determine the toxicity and pharmacology of the agent. Possible age-related differences in effects shall also be taken into account. Pharmacological studies will evaluate the blood kinetics by appropriate measurement in vitro.

CHILDREN'S HOSPITAL OF LOS ANGELES (N01-CM0-7467)

This contract supports a Phase I study of the agent AZQ in pediatric patients. This study is expected to enroll 25-30 patients at various doses to determine the toxicity and pharmacology of the agent. Possible age-related differences in effects shall also be taken into account. Pharmacological studies will evaluate the blood kinetics by appropriate measurement in vitro.

CINCINNATI, UNIVERSITY OF (N01-CM8-7222)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

CLINICA NEUROCHIRURGICA DELL UNIVERSITA (DI PAVIA, ITALY) (N01-CM6-7056)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is being phased out during this fiscal year.

COLLABORATIVE RESEARCH (N01-CM0-7358)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

COMMUNITY BLOOD AND PLASMA SERVICE (N01-CM9-7196)

The purpose of this contract is to provide quadruplet units of HL-A typed platelets to patients in the NIH. The use of HL-A matched platelets decreases the risk of sensitization of production of antibodies to white cells and platelets in patients who require long-term hematologic support. This contract will be administered by the Clinical Center beginning in Fiscal Year 1982.

CONTROL DATA CORPORATION (N01-CM6-7107)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors.

CORDOVA CHEMICAL COMPANY (N01-CM1-7490)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The major effort (approximately 90%) of this contract is devoted to the preparation of large quantities of materials, in the multi-kilogram range, requiring pilot plant facilities.

CORNELL UNIVERSITY (N01-CP8-5652)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is evaluating ultrasound as a technique for assessing body fat and muscle thickness. This contract is complete and final report has been submitted.

DUKE UNIVERSITY MEDICAL CENTER (N01-CM1-7477)

The Neuropathology Department of this institution functions as a neuropathology coordinating center, providing neuropathologic support for the clinical trials conducted by the Brain Tumor Study Group (BTSG). This center has the responsibility for receiving both the surgical and autopsy material and providing the final pathology diagnosis on all patients randomized to the BTSG protocols. In addition, the center conducts and reports special studies correlating various histologic features of brain tumors with the natural course of the disease, effect of treatment and various diagnostic and follow-up procedures.

DUKE UNIVERSITY MEDICAL CENTER (N01-CM6-7010)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is being phased out during this fiscal year.

DUKE UNIVERSITY (N01-CM0-7436)

This Phase I/II clinical trial, utilizing human lymphoblastoid interferon, has been recently activated. It is estimated that the Phase I and II portions of the task will be completed in less than two years.

ELKINS-SINN, INC. (N01-CM6-7103)

The objectives of this contract are to provide facilities and capabilities for the production of parenteral investigational dosage forms for the Division of Cancer Treatment. The contractor is responsible for conformity to FDA Current Good Manufacturing Practices and is responsible for completing all required analytical testing on each product prepared. All products are packaged, labeled and shipped to the National Cancer Institute for subsequent redistribution to clinical investigators.

EMMES CORPORATION (N01-CM8-7193)

The EMMES Corporation provides the statistical support for the Gastrointestinal Tumor Study Group. They assist in the design of protocols, perform statistical analyses of studies, and assist investigators in preparing manuscripts presenting the data.

EMORY UNIVERSITY (N01-CP8-5651)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is evaluating the use of CT scans to measure body fat and muscle mass.

ENERGY, DEPARTMENT OF (Y01-CP8-0207)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is using the unique neutron activation facilities of the Brookhaven Laboratory to study body fat and muscle components in cancer patients.

ENVIRO CONTROL, INC. (N01-CM0-7332)

The objective of this project is to develop and maintain a systematic literature surveillance effort to identify published compounds which warrant acquisition based on their structural characteristics and biological properties. This contract is monitoring a broad base of chemical, biochemical, biological and patent literature to identify compounds for potential acquisition or task order synthesis.

FLORIDA, UNIVERSITY OF (N01-CM9-7320)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of the lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status. In addition, this institution is evaluating the effect of nutritional intervention on the pharmacology of antineoplastic drugs.

FLOW LABORATORIES, INC. (N01-CM6-7088)

This resource contract provides the DCT with storage and distribution capabilities for the large volume of investigational and commercial drugs used in clinical trials. Approved orders for clinical drugs are packaged and shipped to destinations around the world. The contractor also provides a computerized inventory management system. This assures the proper rotation of stock, an adequate lead time to obtain new supplies of drugs and the prompt removal of expired materials.

FLOW LABORATORIES, INC. (N01-CM9-7254)

This contractor furnishes the NCI with facilities and services for the storage and distribution of synthetic chemicals, bulk chemical drugs and crystalline natural products. Samples are weighed, packaged and shipped to contract screening laboratories and also to various domestic and foreign research laboratories. The contract also provides for the maintenance of accurate inventory records. This is an on-going operation of general Developmental Therapeutics Program utility.

FLOW LABORATORIES, INC. (N01-CM0-7370)

This contract is concerned with the development of a practical scale-up procedure to manufacture 50 billion units of human fibroblast interferon during the two year period of the contract. The interferon will be 1×10^7 units/mg of protein.

FOX CHASE CANCER CENTER (N01-CM9-7314)

This contract provides for the support of a clinical neutron therapy program at the University of Pennsylvania--Fox Chase Cancer Center using a DT generator developed under the direction of the University of Pennsylvania in part through grant support from NCI. The addition of Fox Chase Cancer Center to accommodate the DT generator will be provided by this institution. The A/E subcontract was awarded in May, 1980. Fabrication of the DT generator has been completed and the gantry was shipped in March, 1981. The facility will be completed in mid-1981 and testing of the equipment will begin immediately thereafter. Patient treatments should begin in the latter part of Fiscal Year 1981.

FOX CHASE CANCER CENTER (N01-CM0-7330)

The capability for evaluating chemical compounds for radiation protecting properties is provided by this resource. Various physico-chemical parameters are determined on a large number of compounds representing a wide range of chemical structures. Compounds showing potential radioprotector characteristics will undergo in vitro testing to evaluate their cytotoxicity and degree of radiation protection using mammalian cell cultures. Compounds which appear to be superior to the standard - WR-2721 - will be evaluated in vivo, using mice and/or rats. The effect of the compound in modifying the response to radiation will be measured in three different tumor systems (from the DCT panel of mouse tumor screens as stated in the Treatment Linear Array for Radioprotectors), each measured by a separate endpoint. The endpoints will include: the regrowth delay of tumors, tumor cell survival and the modification of the radiation dose required for curing 50% of the tumors. All testing will be compared with the standard WR-2721. The contract also provides for the option of assessing potential long-term tissue injury by performing histopathology on a limited number of tissues and compounds.

FRED HUTCHINSON CANCER CENTER (N01-CM0-7445)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

FRED HUTCHINSON CANCER CENTER (N01-CM0-7336)

A member of the Lung Cancer Study Group (LCSG): The LCSG is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a natural history registry, with new surgical and chemopreventive trials to start patient accrual in 7/81. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of postoperative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. New protocols proposed for activation by the group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of the LCSG have been accepted in four major journals.

GEORGE WASHINGTON UNIVERSITY (N01-CM0-7446)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

GEORGETOWN UNIVERSITY (N01-CM0-7437)

This study is a Phase I and II evaluation of human leukocyte interferon. Currently, patients are being accrued at an adequate rate and laboratory studies to define the role of interferon on the immune system are also being performed.

GEORGETOWN UNIVERSITY (N01-CM1-7501)

This contract is for pathologic support of the clinical trial of chemoprevention. This pathology reference center reviews cytology and culposcopy directed biopsies of the cervix.

GEORGETOWN UNIVERSITY (N01-CM6-7094)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

GEORGETOWN UNIVERSITY (N01-CM6-7110)

This contract supports a comprehensive program for research in the treatment and care of patients with gastrointestinal cancer. The program includes a fully staffed and functioning GI unit, with participation by the surgery, radiology, clinical, pathology, and medical departments. Specifically, the GI research program includes studies in: early detection, staging, new and established anticancer agents, combinations and multidisciplinary approaches, pharmacology, evaluation of markers and professional training. A new major effort is devoted to the study of implications of nutritional deficiencies and symptomatic manifestations of malignancies, and efforts to correct them. Specific areas of investigation being pursued include development of drug regimens in gastric cancer, introduction and study of a new nitrosourea targeted towards gastrointestinal cancer, evaluation of effect of plasmapheresis on tumor growth, and efficacy of heroin on pain control.

GEORGETOWN UNIVERSITY (N01-CM8-7194)

This contract provides operations office functions for the Head and Neck Contracts Project, the Lung Cancer Study Group, the Gastrointestinal Tumor Study Group, and the Intergroup Testicular Cancer Studies. Functions include coordination of protocol design, forms design, quality control of data, all correspondence, randomization, writing of minutes of meetings, and editing and preparation of meeting agenda which include reports on clinical trials. This contract is being recompeted, with award scheduled for September 1981.

GEORGETOWN UNIVERSITY (N01-CM9-7208)

This contract is designed to conduct Phase I and Phase II studies with new anti-cancer drugs sponsored by the DCT. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon.

GEORGETOWN UNIVERSITY (N01-CM9-7310)

This contract is designed to carry out Phase II studies in gastric and pancreatic cancer. The major areas included under the contract are: (1) systematic investigation of new agents in gastric and pancreatic cancer; (2) development of new combinations of agents; and (3) detailed pharmacologic evaluation of single and combined agents.

HARLAN INDUSTRIES (N01-CM0-7362)

This primary genetic center produces a variety of outbred, inbred and hybrids of inbred rodents. All production activities are affected in a closely controlled environment. All foundation colonies are rederived from NIH stock and maintained in associated flora isolators. All expansion colonies are maintained in the barrier environment.

HARLAN INDUSTRIES (N01-CM5-0591)

This contract furnishes approximately 234,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or production colonies. Fixed-price contract # 263-81-C-0095.

HARLAN INDUSTRIES (N01-CM7-7168)

This primary genetic center establishes associated flora foundation colonies from which pedigreed expansion colonies are propagated into barrier rooms. The foundation colonies are rederived from stock furnished by the NIH repository. Rederived expansion colonies are maintained in the barrier environment. Athymic (nude) mice on the Swiss background are also produced under this contract. This contract was terminated during the spring of 1981. This effort will not be recomputed.

HARLAN INDUSTRIES (N01-CM9-7242)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract also furnishes breeding animals for large-scale production colonies. Major emphasis is upon the production of C3H/HeN MTV+, DBA/8, BALB/c-CMC, and CD8F1 mice.

HARLAN INDUSTRIES (N01-CM9-7243)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the primary genetic centers.

HARVARD COLLEGE (N01-CB6-4001)

The objective of this contract is to see if immunotherapy, using antibody to a specific tumor antigen, sustains clinical remission in outbred cats with naturally occurring thymic lymphoma. Cats with chemotherapy-induced complete remission of thymic lymphoma are randomly assigned to no further therapy or treatment with antiserum against feline oncornavirus associated cell membrane antigen (FOCMA). Animals are monitored clinically and immunologically.

HAWAII, UNIVERSITY OF (N01-CM3-3747)

This contract provides for the collection of 3 to 5 lb. plant samples from the indigenous and exotic higher plants of the Hawaiian Islands and Pacific area and the recollection of large samples of those that are reproducibly active in the Developmental Therapeutics Program (DTP) tests. Plants so obtained are shipped to the extraction contract, Raltech Scientific Services, Inc., and the extracts tested at one of our contract screeners. Larger recollections are shipped to the individual fractionators as assigned by DTP. This contract will not be recompeted because it was not approved for recompetition due to budgetary restrictions.

HAZLETON LABORATORIES, INC. (N01-CM6-0125)

This contract provides 150 beagle hounds for toxicological assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor. Fixed-price contract # 263-80-C-0014.

HAZLETON LABORATORIES, INC. (N01-CM8-7156)

This contract provides for the supply of dogs for leukocyte transfusion and bone marrow autograft experiments. The specific experiments underway seek dose-response relationships in leukocyte transfusion of leukopenic dogs with pseudomonas sepsis, and examine the bone marrow stem cell dose, using CFU-C, to reconstitute hemopoiesis after marrow ablative chemo- or radiotherapy. In addition, experiments are underway to explore ex vivo removal of immune of immune blocking factors using a canine mammary carcinoma model. By passing plasma of such dogs over an adsorption column containing staphylococcal A protein, immune complexes are removed and tumors are observed to regress.

HAZLETON LABORATORIES, INC. (N01-CM9-7217)

The objectives of the contract are: (1) to obtain comparative data on the response of nonhuman primates to known rodent carcinogens and to materials suspected of being carcinogenic in man; (2) to evaluate the long-term effects of antineoplastic and immunosuppressive agents which are being used clinically; (3) to obtain model tumor systems in primates for evaluation of new and established antitumor agents; (4) to make available normal and tumor-bearing animals for pharmacologic, toxicologic, biochemical, and immunological studies; (5) to develop biological markers and diagnostic tests for detecting preneoplastic changes as well as frank neoplasia; (6) to develop models for prevention of carcinogenesis, especially for protecting against potent primate carcinogens and against the carcinogenic effects of clinically useful antitumor agents; and (7) to maintain a breeding colony of various species of primates so that these animals may be readily available for use. All work is carried out in close cooperation with the Laboratory of Chemical Pharmacology, DTP.

HEALTH RESEARCH, INC. (N01-A10-2657)

This contract was initiated for a two year period by the National Institute of Allergy and Infectious Diseases, and is funded by the Division of Cancer Treatment. The objectives of the contract are: (1) to develop standard preparations of polyribonucleosinic-polycytidylic acid-poly-L-lysine carboxymethylcellulose (polyICLC), and interferon inducer; (2) to characterize its physical properties; (3) to investigate reproducibility of poly ICLC made with components from various sources; (4) to study the effects of alterations in the formulation; (5) to assay the purity of components; (6) to investigate formulation without carboxymethylcellulose; (7) to evaluate various modified poly IC and poly ICL complexes for interferon induction capabilities and toxic effects; in in vitro cell culture systems, mice and sub-human primates; and (8) to develop in vitro and/or in vivo assays for interferon induction that would be simpler and less expensive than using Rhesus monkeys.

HEALTH RESEARCH, INC. (N01-CB6-4007)

This contract has recently been transferred from DCBD. It consists of the clinical assessment of tumor associated antigen effectiveness in patients with resectable lung cancer. Patients are no longer being accrued, but currently are being followed to determine the value of antigen treatment.

HEALTH RESEARCH, INC. (N01-CM5-7034)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

HEALTH RESEARCH, INC. (N01-CM7-7101)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The contractor carries out the service by performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers. This contract terminated January 31, 1981. New award was made to Northwestern University.

HEALTH RESEARCH, INC. (N01-CM9-7311)

The objective of this program is to evaluate in a Phase II study, photoradiation therapy as a means of local treatment of various malignancies in man. It is planned to determine its scope and limitations and especially to identify situations where it may offer a unique advantage over existing therapies as a treatment for patients who have failed other modalities. Photoradiation therapy involves irradiating hematoporphyrin derivative, which accumulates in malignant tissue, with appropriate laser light in the presence of oxygen. This process generates singlet oxygen, a highly toxic substance.

HEALTH RESEARCH, INC. (N01-CM0-7410)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

IIT RESEARCH INSTITUTE (N01-CM9-7213)

This contract provides assistance to the Drug Evaluation Branch, DTP, staff in monitoring and evaluation of test data and the follow-up of materials demonstrating activity in the primary in vitro and in vivo screens. The contractor participates with staff in the expediting of the scheduling of testing and the evaluation of data on those materials which are recommended for testing in the panel of antitumor test systems. Design of data files used as management tools by Developmental Therapeutics Program staff has been provided and the coordination of the data input to these files continues to be provided by the contractor. These files provide a tracking system for the status of drugs in the Linear Array from decision point 2A and beyond.

IIT RESEARCH INSTITUTE (N01-CM9-7316)

Crude natural products, purified fractions of confirmed in vivo natural products, and synthetic materials are tested for efficacy in the P388 pre-screen tumor system. Upon request, materials are tested in the complete tumor panel. These are L1210, Lewis lung, B16 melanoma, colon 38, CD8F1 mammary, and colon, lung, breast xenograft systems. Testing is conducted at the 46,000 P388 test equivalent level.

IIT RESEARCH INSTITUTE (CM0-7359)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

ILLINOIS, UNIVERSITY OF (N01-CM9-7259)

The objective of this contract is to do a worldwide survey of all the natural products literature, identifying new structures and reports of specific biological activity which may be related to cancer, and reports of biological activity of plant and animal extracts. This allows us to select compounds for testing in our NCI program.

ILLINOIS, UNIVERSITY OF (N01-CM9-7295)

This contract provides for the fractionation of confirmed active plant extracts and fermentation products in order to isolate in pure state and identify the active compound(s). Plant materials used in this work are obtained, for the most part, through the United States Department of Agriculture.

ILLINOIS CANCER COUNCIL (N01-CM0-7415)

A member of the Lung Cancer Study Group (LCSG): The LCSG is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a natural history registry, with new surgical and chemopreventive trials to start patient accrual in 7/81. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of postoperative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. New protocols proposed for activation by the Group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of the LCSGroup have been accepted in four major journals.

INDIAN CANCER RESEARCH INSTITUTE (PL-480 - AGREEMENT NO. NIH-01-010-1)

This is a PL-480 agreement for the collection, identification, preparation of crude extracts, and testing of these extracts in the P388 pre-screen tumor system. In addition, synthetic materials that are synthesized in India, are tested in the P388 pre-screen. This requires the establishment of an animal breeding colony to supply the host animals and a screening laboratory. Both are situated in Bombay. The P388 testing is conducted in accordance with the established Drug Evaluation Branch, DTP, protocol.

INDIANA UNIVERSITY FOUNDATION (N01-CM1-7475)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

INFORMATION MANAGEMENT SERVICES, INC. (N01-CM1-7349)

The purpose of this contract is to provide data management and processing which enables simple and rapid retrieval of clinical information related to the patient data base provided by the participating group of contractors, the Brain Tumor Study Group, who enter patients on study according to specified protocols for treatment of brain tumors.

INFORMATION PLANNING ASSOCIATES, INC. (N01-CM7-7104)

Information Planning Associates, Inc. (IPA), provides technical assistance and support services in the area of investigational drug regulations. Information is gathered and assembled for the preparation of Investigational New Drug Applications (IND's). This includes screening information, animal toxicology, chemistry, bibliographic information, drug labeling and the clinical protocol. This information is submitted to the FDA and an IND is awarded. This contractor then maintains the files by amending information as necessary. IPA is also involved with the preparation of IND annual reports, the establishment of drug master files, the distribution of clinical brochures, and the dissemination of adverse drug reaction information.

INSTITUT JULES BORDET (N01-CM5-3840)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Institut Jules Bordet, Cancer Center of the University of Brussels, Belgium. The program is designed to foster close collaboration between European and United States investigators in the development and application of new clinical anticancer drugs and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer treatment. In addition to the information activities, an animal tumor test program is maintained for the more detailed evaluation and development of selected new compounds, in accordance with NCI interests.

INSTITUT JULES BORDET (N01-CM0-7350) (FORMERLY N01-CM5-7040)

Synthetic materials and crude natural products collected in northern Europe are routinely tested in accordance with the established P388 in vivo protocol. When deemed necessary, materials that originated in the U.S. or other countries are sent to this laboratory for testing. Secondary evaluation in the B16 melanoma, colon 38, L1210, or Lewis lung tumor systems -- according to established protocols -- is conducted upon request of Drug Evaluation Branch (DEB), DTP, staff. Upon request of DEB staff, schedule dependency or more detailed testing is requested of materials of potential clinical interest. Testing is currently being conducted at a level of approximately 11,000 L1210 test equivalents per year.

INSTITUTE OF CANCER RESEARCH (N01-CM4-3736)

This project involves (1) validation of human tumor xenografts as models for cancer chemotherapy; (2) the use of human tumor xenografts and transplantable mouse tumors for testing new compounds of interest to DCT, NCI; (3) toxicology, pharmacology, and initial clinical trials of new drugs developed in this project; and (4) studies of the biochemical basis for treatment response or failure aimed at the design, synthesis, and detailed evaluation of new drugs.

INSTITUTE OF CANCER RESEARCH (N01-CM7-7139)

The objective of this project is to design, synthesize and evaluate nitroimidazoles and related substances that will selectively radiosensitize hypoxic cells in combination with radiotherapy. The compounds are evaluated for electron affinity, cytotoxicity, lipophilicity and in vitro sensitizing efficacy. The focus of this project is to develop a radiosensitizer with less neurotoxicity than misonidazole.

IOWA, UNIVERSITY OF (N01-CM0-7303)

This contract provides services involving dosage form development and manufacture of investigational drugs for subsequent clinical evaluation. Compounds to be formulated are selected and provided by the NCI. The contractor has produced primarily sterile freeze dried injectable products, infusion fluids, and tablets under this contract. However, the contractor has the capability to produce a wide variety of pharmaceutical dosage forms. The contractor is responsible for completing required analytical and safety tests on each product as well as monitoring the stability of the dosage form at recommended and elevated temperatures. All products are packaged, labelled and shipped to the NCI for subsequent redistribution to clinical investigators.

IOWA, UNIVERSITY OF (N01-CM0-7334)

This contract provides capabilities to chemically characterize peptides, proteins and glycoproteins that may be used experimentally and/or clinically to modify tumor growth. Assay methods are developed to analyze the substance in bulk, dosage form and common pharmaceutical vehicles. Studies include determination of amino acid composition, molecular weight, isoelectric point, terminal sequence and development of suitable immunological measurement (radioimmunoassays, etc.) and suitable biological assays.

IOWA, UNIVERSITY OF (N01-CM0-7412) (FORMERLY N01-CM7-7176)

The major objective of this fermentation contract is the preparation of novel antibiotics using the fermentation techniques of biotransformation and co-metabolism. This effort is to convert materials which have solubility, toxicity or marginal activity problems to more active antineoplastic agents. A multitude of unique organisms is isolated and used to obtain these microbial conversions in an effort to obtain antineoplastic agents.

IOWA, UNIVERSITY OF (N01-CM1-7476)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

IOWA, UNIVERSITY OF (N01-CM9-7319)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of the lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM0-7338)

A major effort in breast cancer has been through this contract. It has dealt primarily with adjuvant therapy of resectable disease, and its results have received worldwide attention. The Istituto has recently shown an improved overall survival for premenopausal patients treated with CMF. They also recently reported that 12 months of CMF is no more effective than 6 months. A re-analysis of disease-free survival among postmenopausal patients showed a clear advantage for patients receiving an average >75% drug dose compared to those with <75% drug dose.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM3-3714)

The major thrust of this contract has been the multidisciplinary approach to breast cancer at all stages. Completed studies in the very important follow-up phase include the "CMF combination chemotherapy versus placebo following radical mastectomy for Stage II carcinoma" and the "CMF 12 versus six months of treatment" study in the same stage. More recent studies include a detailed analysis of estrogen receptor and pathologic data in influencing relapse rate in premenopausal women treated with CMF. Postmenopausal women under 65 are being treated with a delayed intensification schema versus conventional CMF/AV treatment, and older women with tamoxifen. Work is continuing with innovative protocols at all stages including locally inoperable and advanced breast cancer. In other disease areas, this contract has participated within the Gastrointestinal Tumor Study Group in colon adjuvant studies and is developing new protocols to test concepts of late intensification in testicular, small cell of lung, diffuse histiocytic lymphoma, and breast cancer.

ISTITUTO NAZIONALE PER LO STUDIO E LA CURA DEI TUMORI (NO1-CM4-3726)

This contract represents utilization of a unique resource of both advanced and early patients with malignant melanoma for various controlled clinical trials. The Project Director is the Chairperson of the International Group for the Clinical Study of Melanoma. There are 27 collaborating institutions including Eastern and Western Europe, Australia (Sydney), and South America (Montevideo). Investigators have surgical, pathologic, chemotherapeutic and immunotherapeutic expertise. A clinical trial, to determine the value of adjuvant DTIC, BCG, DTIC + BCG or no further therapy following lymph node dissection in patients with nodal melanoma metastases or Stage I lesions of the trunk, is currently in progress. The study will determine whether the above adjuvant therapies increase the disease free interval for those patients. In addition, a trial of DTIC, DTIC + BCG, or DTIC + C. parvum in advanced malignant melanoma is also in progress. This study will determine the response rates and duration of response for patients with metastatic melanoma.

JAPANESE FOUNDATION FOR CANCER RESEARCH (N01-CM2-2054)

The objective of this contract is the maintenance of a chemotherapy liaison office at the Japanese Foundation for Cancer Research in Tokyo. The program is designed to foster close collaboration between Japanese and United States investigators in the development and application of new clinical anticancer drugs, and in the exchange of preclinical experimental and clinical scientific knowledge and materials requisite for maximum progress in cancer therapy. A small testing facility is also maintained for the screening and further evaluation of selected new compounds.

KANSAS, UNIVERSITY OF (N01-CM0-7304)

This contract provides research capabilities in the area of dosage form design and development. Formulation problems not amenable to the usual solubilization and/or stabilization methods are investigated. The contractor has particular expertise in the application of molecular complexes and reversible derivatives to improve solubility. Pilot batch preparation and chemical evaluation of these novel formulations are carried out under this contract.

KANSAS, UNIVERSITY OF (N01-CM9-7272)

This contract is designed to conduct Phase I clinical studies with new anti-cancer drugs sponsored by the DCT or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

KAROLINSKA INSTITUTE (N01-C37-4144)

The objective of the contract is to demonstrate the existence of specific human tumor-associated antigens and to show in vitro increased responses to such antigens after in vitro immunization with autochthonous tumor. Human tumor specimens (carcinoma of the lung, astrocytomas, osteo- and soft tissue sarcomas) will be characterized and fractionated for tumor and lymphoid cells. Cytotoxicity tests will be performed with biopsy target cells using blood lymphocytes and tumor infiltrating lymphocytes, as effectors, both directly at harvest and after cocultivation in vitro. Attempts will be made to characterize the nature of the effector cell. This contract will expire in September 1981.

KENTUCKY, UNIVERSITY OF (N01-CM0-7381)

This contract is assigned difficult dosage form development projects not amenable to the usual solubilization and/or stabilization approaches. This contractor has particular expertise in the application of reversible derivatives (prodrugs) to improve drug solubility. Pilot batch preparation and chemical analysis of these novel formulations are carried out under this contract.

KENTUCKY, UNIVERSITY OF (N01-CM6-7058)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

KING ANIMAL LABORATORY (N01-CM1-7499)

This procurement contract is designed to furnish 156,000 six-week old first generation hybrid mice for Developmental Therapeutics Program contract studies. Fixed-price contract # 263-81-C-0103.

KOLLING INSTITUTE (N01-CB8-4251)

The objective of this contract is to conduct research designed to functionally characterize factors produced by and/or induced by tumors, which impair macrophage function and/or inflammatory responses in vivo and in vitro. Attempts will be made to evaluate the nature, extent, mode of action, and duration of impairment of macrophage function and inflammatory responses produced directly or indirectly by tumor cell products. Tumors will be of mouse, rat, and human origin. The effects of such factors on monocytes, stimulated and activated macrophages, and on tumor-associated macrophages will be compared. In addition, studies will be undertaken to assess the significance of such factors with respect to tumor growth in animals. This contract is due to expire in September, 1981.

KYOWA HAKKO KOGYO CO., LTD (N01-CM8-7190)

This contract is to evaluate the ability of plant cells to grow and to produce the antineoplastic agent in submerged fermentations. Often plants which produce antineoplastic agents are found in foreign countries. In order to insure supply of these plants, one must be able to cultivate them or find worldwide sources. Weather and political conditions often make it prohibitive to obtain plant material in sufficient quantity to isolate adequate amounts of the antineoplastic agent for NCI evaluation. The contract is evaluating the possibility of propagating plant cells in submerged culture to produce antineoplastic agents of interest. This would insure local supply of such agents and allow NCI to

KYOWA HAKKO KOGYO CO., LTD (N01-CM8-7190) (CONTINUED)

move quickly to obtain these materials. Eight plants of interest to NCI have been assigned and one of these has been propagated and produces the compound of interest. This contract will not be recompeted because of budgetary restrictions.

LABORATORY SUPPLY COMPANY, INC. (N01-CM5-0577)

This contract furnishes approximately 234,000 CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are being furnished from genetic centers and/or rodent production colonies. Fixed-price contract # 263-81-C-0102).

LABORATORY SUPPLY COMPANY, INC. (N01-CM9-7244)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigators. This contract also furnishes breeding animals for large-scale production colonies. All activities are performed in bio-containment environments. The breeding stock is received from primary genetic centers.

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (N01-CM7-7165)

This primary genetic center has as its objectives the development of associated foundation colonies of inbred rodents. Pedigreed animals are derived via hysterotomy and foster-suckled in germfree isolators. Selected pedigreed offspring are artificially contaminated with pure cultures of non-pathogenic organisms and are developed as pedigreed expansion colonies in isolators. Offspring from this second stage are issued to rodent production centers which, in turn, produce large-scale production colonies. The methods commonly accepted as best practice are followed with respect to environmental controls and microbiological monitoring. A small-scale production colony is maintained in order to provide limited numbers of rodents for special research and testing studies.

LITTON BIONETICS, INC. (N01-CM0-5724)

The major objective of this contract is to provide a well-equipped animal facility to satisfy the needs of various laboratories in the Developmental Therapeutics Program. Services provided are utilized for studies relating to the effects of drugs in the production of tumors, the study of metabolites of various drugs, the production of antisera and studies of the pathogenesis of leukemia. The facility can maintain 3,500 mice, 30 rabbits, 20 guinea pigs, 50 rats, 15 goats, 4 gibbon apes and 10 dogs with essential veterinary care available 365 days a year. The contract also provides technical assistance for performance of routine procedures and professional assistance for surgical procedures and post-mortem examinations.

LITTON BIONETICS, INC. (N01-CM0-7326)

The major objectives of this contract are to prepare and supply large quantities of concentrated primate and putative human type C RNA tumor viruses.

LITTON BIONETICS, INC. (N01-CM0-7347)

The major objectives of this contract are: (1) the use of radioimmunoassays to screen human B lymphoblast cell lines and cell clones for viral structural protein expression; (2) the use of ELISA assays to detect antibodies against a putative human virus in serum from leukemic patients and normal donors; (3) to test culture fluids from short-term and long-term cultured cells for the presence of viral DNA polymerase activity; (4) to supply freshly separated hematopoietic cells from normal and leukemic peripheral blood, cord blood and bone marrow, and cells from short and long-term cultures of lymphoid and myeloid cells; (5) to test cultured cells for colony formation in semisolid media; and (6) to test sera from subhuman primates and from humans for humoral antibodies to type C RNA tumor viruses and to provide storage facility for such sera.

LITTON BIONETICS, INC. (N01-CM6-7067)

This contract supports the efforts of the Surgery Branch, COP, to store serum samples on all Surgery Branch patients. Approximately 100,000 serum samples are stored and must be available for retrieval for clinical studies. In addition, this contract supports the production of T-cell growth factor for use in experimental and clinical immunotherapy protocols. This will continue without change.

LITTON BIONETICS, INC. (N01-CM8-7169)

This contract assists the intramural scientists in the Clinical Oncology Program in the storage and maintenance of laboratory animals. The facilities of the Clinical Center are extremely limited in the availability of space and personnel for laboratory animal handling, and this investigative resource is commonly available through a contract mechanism. The contract combines the animal holding and transport needs of all Branches in Clinical Oncology into one support contract. The contractor maintains, feeds, and transports the animals but does not conduct research. Tumored animal models are also provided.

LITTON BIONETICS, INC. (N01-CM8-7187)

The major objectives of this contract are: (1) to purify and test factors that promote growth and differentiation of myelogenous leukemic cells and T cells; (2) to purify the envelope and internal structural proteins of type C RNA tumor viruses and the use of competition radioimmunoassays for detection of these viral antigens; (3) to prepare monoclonal antibodies against the purified structural proteins and the use of these antibodies for the detection of related proteins in human tumor cells; (4) to prepare and supply radiolabeled RNA and cDNA from selected type C RNA tumor viruses for use in the detection of type C viral information in human leukemic cells; and (5) to provide structural analysis of purified proteins for comparison and analysis.

LITTON BIONETICS, INC. (N01-C07-5380)

The Government-owned contractor-operated Frederick Cancer Research Center (FCRC) serves as a multi-faceted contract involving the following activities for the DCT:

Biologic Markers Program (BMP)

A group of 10 professionals and their supporting staff has conducted an ongoing program of investigation of new biochemical markers that correlate with the occurrence and clinical course of various types of cancer in patients. This has involved isolation, purification and characterization of compounds qualitatively or quantitatively unique to cancer cells and measurable in serum, urine or exudates. These compounds have included DNA-binding proteins, ectopic hormones, isoenzymes and abnormal degradation products of normal proteins. Because of changes in program emphasis, this biological markers project of the FCRC contract is being phased out and is expected to terminate in about the middle of FY 1982.

Chemotherapy Fermentation Laboratory (CFL)

The DCT supports a fermentation pilot plant facility at the FCRC for the large-scale production and isolation of microbial products of interest to the Chemotherapy and Biological Response Modifiers Programs. Facilities are also available for the production of other natural products. This year kilogram quantities of daunomycin were produced in addition to interferon for amino acid sequencing studies by Dr. Chris Anfinsen of the NIAMDD. Fermentation and development is underway to increase yields of largomycin FII and toyocamycin. These materials will be produced in larger quantities for use in the program. Two novel anti-neoplastic agents have come from the earlier research effort and larger amounts of material will be produced for NCI evaluation.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CM9-7250)

This contract provides a multidisciplinary approach to drug development. The three main tasks include: (1) the collection and screening of antineoplastic agents obtained from Southern Europe; (2) the screening of immunochemotherapeutic agents; and (3) pharmacologic studies of new agents with emphasis on the relationship of pharmacokinetics with in vivo antitumor effects. This contract expired on March 31, 1981.

"MARIO NEGRI" INSTITUTE OF PHARMACOLOGICAL RESEARCH (N01-CP8-5604)

This is a preclinical contract testing agents for anti-anorectic activity in an animal tumor model. Weekly positive results were observed with cyproheptidine, but many other agents have given negative results.

MARSHALL RESEARCH ANIMALS, INC. (N01-CM6-0123)

This contract provides 150 beagle hounds for toxicological assays. These animals are raised under controlled conditions in accordance with the specifications delineated in the contract. Breeding animals are supplied by the contractor. Fixed-price contract # 263-80-C-0016.

MARYLAND, UNIVERSITY OF (N01-CM4-3748)

Under this support services contract, the University of Maryland at Baltimore, provides clinical and laboratory facilities, administrative support, and ancillary support services and personnel for the clinical and preclinical research program of the Baltimore Cancer Research Program. These activities include the Inpatient and Outpatient Departments, Cell Component Therapy, Clinical Research Pharmacy, Infection and Microbiological Research Sections, Laboratory of Clinical Biochemistry, and the Laboratory of Molecular Biology.

MARYLAND, UNIVERSITY OF (N01-CM8-7223)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

MASON RESEARCH INSTITUTE (N01-CM0-7325) (FORMERLY N01-CM5-7030)

The purpose of this contract is to develop new in vivo tumor models with predictive value in selecting clinically effective drugs, to establish and maintain in serial transplantation human tumor cell lines in nude mice as assay systems, to validate human tumor xenografts as screening models, to develop other in vivo tumor models as indicated by Program needs, to maintain the assay developed, and to conduct special non-routine testing in assays other than the tumor panel upon specific request. The subrenal capsule (SRC) assay was developed and validated under this contract.

MASON RESEARCH INSTITUTE (N01-CM6-7011)

This contract supports the Clinical Oncology Program of the DCT with computer programming expertise for the development of clinical information systems and with data technician services for the maintenance and utilization of these systems. The contract also provides data management services for statistical center activities of the Biometric Research Branch, COP. A wide variety of systems has been developed and are maintained for the Clinical Branches of the DCT.

MASON RESEARCH INSTITUTE (N01-CM8-7164)

This contract has as its major goal the maintenance of approximately 20,000 frozen tumor vials. This contractor furnishes needed tumors to the various DTP screening laboratories, as well as to other research institutions, both domestic and foreign. The tumors are supplied both in vivo and in vitro.

MASON RESEARCH INSTITUTE (N01-CM9-7317)

Employing the P388 in vivo tumor system, synthetics and materials of natural product origin are assayed for efficacy. Materials, which are assigned to the tumor panel, are tested in the B16 melanoma, the colon 38, Lewis lung, L1210 murine tumor systems and the colon, lung and mammary ^{human} xenograft systems. Methodology research is conducted at the request of the Project Officer.

MASSACHUSETTS GENERAL HOSPITAL (N01-CP8-5657)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is studying lipid mobilization and the response to fasting in cancer patients.

MATHTECH, INC. (N01-CM9-7195)

The objective of this contract is to provide a clinical trials monitoring service for clinical trials conducted by the Phase I and II Working Group. This service has two components: (1) to provide a central data management resource for both the Investigational Drug Branch, CTEP, and for the clinical investigators conducting these studies, and (2) to provide a monitoring resource to meet the Food and Drug Administration regulatory requirements and to complement the data management objectives.

MAYO FOUNDATION (N01-CM0-7414)

A member of the Lung Cancer Study Group (LCSG): The LCSG is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a natural history registry, with new surgical and chemopreventive trials to start patient accrual in 7/81. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cytoxan, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of postoperative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. New protocols proposed for activation by the Group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiationtherapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of the LCSG have been accepted in four major journals. _

MAYO FOUNDATION (N01-CM0-7419)

This is one of four new contracts devoted to the development of human tumor clonogenic assay for drug screening. As the first phase of the project, each contractor will be engaged in a pilot study which involves testing 50 unknown compounds against a spectrum of human tumors. Upon the completion of testing, the data will be subjected to statistical analysis. Each contractor is also conducting limited developmental studies, such as comparisons of enzymatic and mechanical tissue disaggregation techniques and comparisons of different media. The objective of these latter studies is to explore ways of improving cloning efficiency and cost efficiency of the system for mass screening.

MAYO FOUNDATION (N01-CM4-3783)

This contract is designed to explore in prospective, randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease; and (3) Phase II evaluation of single agent chemotherapy in previously treated patients. This contract is currently in phase out status.

MAYO FOUNDATION (N01-CM4-3796)

This contract is designed to evaluate a combined modality protocol employing radiotherapy and chemotherapy in the treatment of locally unresectable carcinoma of the pancreas. In addition, an ongoing evaluation of single-agent and combination chemotherapy is currently underway with the G.I. Tumor Study Group. The institution also participates in the protocol testing radio-chemotherapy as an adjuvant to surgically curable pancreatic cancer. This contract is currently in phase out status.

MAYO FOUNDATION (N01-CM5-7033)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract is currently in phase out status.

MAYO FOUNDATION (N01-CM5-7044)

This contract is designed to support Phase II/III studies in solid tumors, by means of prospective, randomized, controlled clinical trials involving the use of combined modalities. This contractor was phased out during FY 1981.

MAYO FOUNDATION (N01-CM6-7123)

This contract provides collaboration in a group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IAi and IBi) compares observation to L-PAM post-operatively. A secondary study involves a histo-pathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patient population. A further joint protocol for other early disease (i.e., FIGO Stage IAii, IBii, IC, and II) separates patients according to presence or absence of macroscopic residual disease post-operatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients with macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate. This contract is currently in phase out status.

MAYO FOUNDATION (N01-CM9-7268)

This contract calls for a Phase II evaluation of therapies in advanced gastrointestinal cancer. More than 175 patients have been entered into this contract during the past year. The role of the soft agar clonogenic assay as a prospective means of selecting chemotherapy will be analyzed in some colon cancers during FY 1981.

MAYO FOUNDATION (N01-CM9-7273)

This contract is designed to conduct Phase I and Phase II studies with new anti-cancer drugs sponsored by the DCT. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon.

MEDICAL RESEARCH COUNCIL (N01-CM8-7171)

The purpose of this contract was to investigate several aspects of the pharmacologic, toxicologic and therapeutic properties of liposome-encapsulated antitumor agents. The agents studied were melphalan, vincristine and platinum-based drugs. Studies were directed toward developing techniques for the production of liposomes homogeneous with regard to size and composition, and to assess chemical stability under various conditions of storage. Initial studies were conducted on the permeability of liposomes to small molecular weight substances in aqueous media as well as in biological fluids such as serum and whole blood; the efflux rate of entrapped substances as a function of liposome composition was also evaluated. In vitro studies were performed on the effects of chemical composition surface charge and particle size of liposomes on their affinity for, uptake by, and interaction with, normal and tumor cells. Comparative studies on the physiologic disposition of free and encapsulated antitumor agents in normal and tumor-bearing animals were performed, in parallel with an evaluation of the effect of variations in the chemical composition, surface charge and particle size of liposomes on their in vivo distribution. Dose-reponse relationships for the liposome-encapsulated antitumor agents as well as the optimal route of administration (ip, iv or im) and dosage schedule were studied. The acute toxicity of the liposome-encapsulated antitumor agents was assessed and compared with that of the free drug in normal and tumor-bearing animals. This contract terminated December 31, 1980.

MELOY LABORATORIES, INC. (N01-CM0-7378)

The purpose of this contract is to produce 50 billion units of clinically acceptable leukocyte interferon. 7.8 billion units have been delivered and the purity is 6×10^6 units/mg protein.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM1-7348)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forward into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM1-7348) (CONTINUED)

these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized as well as insight into the biologic characteristics of the disease entity being investigated.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM8-7224)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM9-7274)

This contract is designed to conduct Phase I and Phase II studies with new anti-cancer drugs sponsored by the DCT. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM0-7337) (FORMERLY N01-CM5-7043)

This contract is for Phase II/III studies to detect useful therapeutic effects of new drugs alone as well as other drugs in combination in solid tumors. The previous contract (N01-CM5-7043) completed a 5-year contract. During the period 1977-1980, a total of 1,159 patients were entered into Phase II trials, and 1,590 patients into Phase III trials for a total of 2,749 entries. Between 2/1/79 and 1/31/80, 233 patients were entered into poly-drug studies. The results of these studies were reported in 56 papers and 19 abstracts.

This group has made significant contributions and has more than complied with the terms of the contract. Some major contributions include studies involving: (1) head and neck cancer, bladder cancer, testicular cancer; (2) Phase II studies of high-dose platinum, pyrozofurin, neocarzinostatin in bladder, prostate and hepatoma; (3) high-dose vs low-dose DDP in breast; and (4) DCNU, DDP+DVA in lung and the Interactive MUMPS-Based Data System for the Clinical Oncology Program to monitor and analyze chemotherapeutic and multidisciplinary treatment protocols for patients with advanced cancer.

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CM0-7337) (CONTINUED)

A new Phase II/III contract was successfully initiated for the period 9/15/80 to 2/14/86. New studies approved or to be submitted include: (1) VAB VI - germ cell tumors; (2) CAP vs sequential chemotherapy in ovarian cancer; (3) cis-platinum + vindesine + bleomycin in esophageal carcinoma; (4) cis-platinum + bleomycin in the treatment of advanced cancer of the cervix; (5) vindesine, cis-platinum and bleomycin vs methyl-G in advanced non-small lung cancer; (6) intensive combination chemotherapy and autologous stem cell transplantation for the treatment of small cell carcinoma of the lung in first relapse; (7) VLB + MTX for advanced urotract tumors; (8) high-dose DDP with mannitol in previously untreated patients with advanced soft tissue sarcomas; (9) cis-platinum + VP 16 in germ cell tumors; (10) randomized study of maintenance vs no maintenance in good risk metastatic germ cell tumors; (11) VP-16 in gastric cancer; (12) prolonged induction with cis-platinum, bleomycin, cytoxan and adriamycin in advanced head and neck tumors; and (13) combined modality therapy of esophageal cancer comparing preoperative CT vs preoperative RT.

MIAMI, UNIVERSITY OF (N01-CM0-7409)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

MIAMI, UNIVERSITY OF (N01-CM6-7093)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MIAMI, UNIVERSITY OF (N01-CM9-7290)

Crude natural product extracts, fractions of confirmed natural products, and synthetic materials submitted in too small a quantity to permit meaningful in vivo testing are routinely tested in the 9ASK astrocytoma in vitro tumor system. Testing in the 9KB in vitro tumor system is limited to the confirmation of materials previously presumptively cytotoxic and fractions of confirmed natural products that were cytotoxic in this system. Testing in the 9PS and/or 9LE in vitro tumor system is limited to fractions of crude natural products that are being monitored in these systems. Protocols for these systems have been established by the Drug Evaluation Branch, DTP, and all testing is conducted in accordance to the appropriate protocol. Approximately 15,000 9KB test equivalents are scheduled per year.

MICHIGAN, UNIVERSITY OF (N01-CM0-7405)

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 200 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

MICHIGAN, UNIVERSITY OF (N01-CM8-7225)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

MICHIGAN TECHNOLOGICAL UNIVERSITY (N01-CM0-7293)

The objective of this project is the rational design and synthesis of effective inhibitors of purine nucleoside phosphorylase (PNPase) and hypoxanthine guanine phosphoribosyl transferase (HGPRTase), key enzymes in the purine salvage pathway. Another target enzyme might be inosinic dehydrogenase, a key enzyme that is rate-limiting in the synthesis of GMP and catalyzes an irreversible reaction.

MICROBIAL CHEMISTRY RESEARCH FOUNDATION (N01-CM5-7009)

The major objective of this contract is the preparation of fermentations of marine, psychophilic and thermophilic organisms. These fermentations are screened against various enzyme and other biochemical screens. Active products are isolated in sufficient quantities to be evaluated at the National Cancer Institute. In addition, various immunogen tests have been developed to evaluate the organisms and their metabolites as potential immunological stimulators specific for cancers. One compound from this work is in Phase II clinical trials in the U.S.A. Another compound has just passed DN2.

MICROBIOLOGICAL ASSOCIATES (N01-CM0-7369)

This contract resource assists in the measurement of leukocyte compatibility in clinical transfusions of leukopenic patients. The contractor performs several leucoagglutinin assays and lymphocyte cross-match studies in order to insure safe and reliable blood component transfusions. These tests are used in the daily management of the NCI leukopheresis program. In addition, the contractor operates a computerized serum storage bank for all patients at NCI which has accessioned over 20,000 samples.

MICROBIOLOGICAL ASSOCIATES (N01-CM9-7246)

This rodent production center supplies inbred rodents for tumor transplantation, for hybrid production, and for compound evaluation studies. Animals are supplied from a colony of four strains of rodents.

MICROBIOLOGICAL ASSOCIATES (N01-CM9-7287)

This contract has as its major purpose serological diagnosis for the presence or absence of infectious mouse ectromelia, LCM, polyoma, M. hepatitis, M. adenovirus, PVM, Sendai, Theiler's (GDVII), K, Rheo 3, Toolan's H-1, and the Rauscher and Maloney viruses. The contractor also carries out ancillary research concerned with the effects of viruses, such as vaccinia and polyoma, on the growth of tumors; interaction of polyoma virus with tumors; anti-body producing capacity of tumor-bearing mice and the effect of the route of injection on antibody levels; and a comparison of the sensitivity of Caesarian-derived and other mice to polyoma virus. This activity furnishes information on the effects of "passenger" agents on the growth of tumors used in the screen and on their effects on drug action. During a contract period of one year, this laboratory performs approximately 68,400 rodent serum and 12,000 tumor fragment diagnostic tests. The contractor also prepares vaccinia virus which is used for immunizing mice against infectious ectromelia.

MID-AMERICA CANCER CENTER (N01-CM0-7353)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

MIDWEST RESEARCH INSTITUTE (N01-CM8-7234)

Midwest Research Institute is the smaller of the two contractors responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological and clinical phases of the Developmental Therapeutics Program. The contractor determines the identity and purity of the compounds by appropriate methods. The contractor also determines solubility, stability and other physical chemical properties to aid in formulation development and selection of storage conditions. Techniques commonly used include elemental analysis, chromatography (paper, thin layer, gas liquid and high-pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry and other methods as needed. Reports of the work performed by the contractor provide data to be included in IND filings with the Food and Drug Administration and for quality assurance monitoring.

MILES LABORATORY, INC. (N01-CM9-7172)

This is a no-cost contract concerned with the joint development of the antitumor agent, chlorozotocin. Under this agreement, the contractor supplies the drug and provides other services to the NCI (at its own expense) in support of NCI-sponsored clinical studies. If warranted by future results, the contractor will prepare and submit a New Drug Application (NDA) to the Food and Drug Administration and will eventually market the drug commercially in the United States.

MISSOURI, UNIVERSITY OF (N01-CM8-7157)

This contract will provide for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from the barrier room expansion colonies and the nude mouse production colonies.

MISSOURI, UNIVERSITY OF (N01-CM9-7211)

This contract monitors the animal production contracts by testing for the presence or absence of Salmonella and Pseudomonas. Samples are received on a scheduled basis from the animal producers and approximately 18,000 fecal samples are tested per year.

MONSANTO RESEARCH CORPORATION (N01-CM9-7255)

This service preparative contract provides for the large-scale synthesis of compounds required for preclinical and clinical studies. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. The effort of this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

MONTEFIORE HOSPITAL (N01-CM1-7474)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CB4-3879)

The goal of this contract is to evaluate the usefulness of neurominidose-treated allogenic AML cells in acute leukemia. Preliminary data suggest that immunotherapy may offer an advantage over chemotherapy alone. Continuation of this contract is planned through June 1984.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM0-7407)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM6-7096)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM9-7275)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the DCT or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

MOUNT SINAI SCHOOL OF MEDICINE (N01-CM9-7294)

The purpose of this contract is to provide in-depth clinical pharmacology studies on agents undergoing Phase I or Phase II clinical evaluation. A reverse phase ion-pair HPLC analysis was developed for methylglyoxal-bis-guanylhydrazone (NSC-32946; Methyl-G). Sensitivity was 30 mg/ml in serum, ascites and pleural fluid, and 300 mg/ml in urine. Ethylglyoxal-bis-guanylhydrazone was used as the internal standard. Using this technique, 29 patients were studied for drug determinations in serum and urine. The drug levels in ascites and pleural effusions were also studied in a small number of patients. The serum drug decay was triphasic with $t_{1/2\alpha} = 20$ min, $t_{1/2\beta} = 3$ hours, and $t_{1/2\gamma} = 100$ hours. Weekly administration resulted in cumulative effects. Methyl-G was rapidly transported into ascites and the ascites drug levels were higher for 1 to 12 hours after drug administration. In patients with normal renal function, 25 to 30% of the drug was recovered in the urine in 24 hours. There was no evidence for metabolism of Methyl-G. A reverse phase ion-pair HPLC analysis was developed for dihydroxy-anthracenedione (DHAD; NSC-279836), that is sensitive to 10 ng/ml. C^{14} -DHAD is available and will be used to study the metabolism of DHAD in patients. Related in vitro studies were performed on the binding of DHAD to DNA as determined by competitive fluorescent polarization. Also, studies on the binding of dichloromethotrexate to albumin were completed. This contract will terminate during this fiscal year; it will be converted to the Task Order mechanism and will not be recompleted.

MUHIMBILI MEDICAL CENTER (N01-CM0-7344)

This contract is to initiate the study of the demography and epidemiology of cancer in albino Africans at the University of Dar Es Salaam. Efforts will be made to establish an experimental model of the skin cancer/albino problem and its prevention with various retinoids or other materials.

MURPHY BREEDING LABORATORIES (N01-CM5-0579)

This contract furnishes approximately 234,000 six-week old CD2F1 (BALB/c female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers. Fixed-price contract # 263-81-C-0100.

NATIONAL ACADEMY OF SCIENCES (N01-CM5-3850)

This Task Order serves to develop: (1) standards for animal care and maintenance; (2) shipping standards for the various species of laboratory animals, standards for nomenclature used to identify stocks and strains of laboratory animals; (3) standards for methodology in maintaining axenic and gnotobiotic animals; (4) standards for animal maintenance in the research laboratory; and (5) laboratory animal procurement standards. These standards are formulated by ad hoc committees whose memberships represent commercial animal production colonies, governmental and academic institutions, commercial laboratories, and non-profit research institutions.

NATIONAL CENTER FOR HEALTH STATISTICS (Y01-CM8-0109)

The objective of this Inter-agency Agreement is to develop, implement and install a completely operational, functioning, computerized, expandable, and exportable PROMIS System into the Baltimore Cancer Research Program clinical cancer treatment research setting.

NATIONAL NAVAL MEDICAL CENTER (Y01-CM0-0103)

The Clinical Oncology Program (COP) is supporting several clinical initiatives of the National Naval Medical Center (NNMC) through an Inter-agency Agreement. Patients are entered into NNMC studies relative to melanoma, carcinoma of the colon, acute leukemia, lymphoma, breast cancer, and carcinoma of the kidney. These studies/projects are done in collaboration with the staff of the COP.

NAVY, DEPARTMENT OF (Y01-CM1-0200)

This contract was for the design of plans for renovation of space in Building 1 (The Tower), and Building 8 of the National Naval Medical Center (NNMC), Bethesda, Maryland, to accommodate the NCI-Navy Medical Oncology Branch. This Branch will take the place of the NCI-VA Medical Oncology Branch and will be staffed by the personnel of the former NCI-VA Medical Oncology Branch. The transfer of clinical responsibilities will occur in July, 1981, and the full transfer of the Inter-agency Agreement from the VA to the Navy will occur October 1, 1981. The consulting architectural firm of Ellerbe, Dalton, Dalton and Newport (Mr. H.C. Allison and staff) conducted numerous meetings with the senior medical and nursing staff of the NCI-VA Medical Oncology Branch and the responsible individuals of the NNMC (headed by Cmdr. J. Smith) conducting the retrofitting of the NNMC to coordinate and plan this project. This led to the design of plans for renovating 6 floors of space (2 in Building 1 and 4 in Building 8) to be coordinated over a 2-3 year period. These plans were completed and received technical approval.

NAVY, DEPARTMENT OF (Y01-CM1-0201)

This contract is to cover the renovation and retrofitting of space in the National Naval Medical Center (NNMC), Bethesda, Maryland, to be occupied by the NCI-Navy Medical Oncology Branch. The NCI-Navy Medical Oncology Branch will begin clinical responsibilities in July, 1981, and the Inter-agency Agreement formerly held with the Veterans Administration will shift to the NNMC as of October 1, 1981. Preliminary to this: (1) a "Memorandum of Understanding" was signed between the Surgeon General of the Navy and the Director, NIH, and the Director, NCI; (2) meetings were held between NCI-VA, other NCI officials, Navy staff and with the Navy consulting architects firm of Ellerbe, Dalton, Dalton, and Newport (see contract Y01-CM1-0200); (3) a draft agreement (prepared by Dr. J. Minna, NCI, and Capt. D. Pasquale, NNMC) governing the interaction of the NCI and the NNMC was approved in principle by the Commanding Officer, NNMC, and the Director, NCI. As part of this contract: (1) the NNMC will provide a completely designed and functional clinical ward of 30 beds (ward 6 West) in their new hospital facility in Bethesda which was completed and had occupancy

NAVY, DEPARTMENT OF (Y01-CM1-0201) (CONTINUED)

January, 1981; (2) two floors of temporary "swing space" in Building 1 (floors 4 and 5) for housing the outpatient clinic, offices, laboratories, and conference rooms; (3) four floors of "permanent" space in Building 8 (floors 3, 4, 5 & 6) to house the same functions; (4) the Navy retrofitting facility (Dept. of the Navy, Chesapeake Division, Naval Facilities Engineering Command, Washington Navy Yard), will supervise the retrofitting and renovation of the space on site and submit monthly reports as to the status of the work. The NCI is providing funds to cover the retrofitting/renovation as detailed in NAFAC plan numbers: 3116145, 3116153, 3116301, 3116302, 3116159, and 3116160. These plans, under contract Y01-CM1-0200, have been prepared by Ellerbe, Dalton, Dalton, and Newport with the medical/laboratory technical input of the NCI-VA Medical Oncology Branch senior staff and are to be retrofitted/renovated to the NCI-VA staff's specifications. The milestones are: bid award date July, 1981; occupancy of Building 1 "swing space" January-April, 1982; and occupancy of Building 8 "permanent space" January, 1984. The cost of the renovation includes contingencies fee of 10% and overhead of 5.5%. The contract is to be a firm, fixed-price award made by the Department of the Navy and the amount will be adjusted after the bid opening and award. This renovation will be part of the larger renovation of the NNMCMC. This allows the NCI to have the lowest cost for renovation, saving the NCI from negotiating a separate, more costly, contract. The contract has been signed and approved by the Director, NCI, and the Commanding Officer, NNMCMC, Bethesda, Maryland.

NEW YORK UNIVERSITY MEDICAL CENTER (N01-CM1-7473)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

NEW YORK UNIVERSITY MEDICAL CENTER (N01-CM9-7321)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of the lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status.

NORTH CAROLINA, UNIVERSITY OF (N01-CM1-7471)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

NORTHERN CALIFORNIA CANCER PROGRAM (N01-CM0-7446)

This is a Phase I evaluation of thymosin fraction 5 and alpha-1. This study is in an early stage and preliminary data are being collected.

NORTHERN CALIFORNIA ONCOLOGY GROUP (N01-CM8-7154)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

NORTHROP INDUSTRIES (N01-CM0-7286)

This contract has as its major purpose serological diagnosis for the presence or absence of murine viruses in rodents. During a contract period of one year, approximately 40,200 virus serology tests are performed.

NORTHWESTERN UNIVERSITY (N01-CM1-7363)

This contract is designed to monitor and maintain genetic control of tumor strains and inbred mouse stocks. This service was established to assure continuous control of the biological materials used in program studies. The contractor carries out the service by performing skin grafts and antigenic studies of mouse strains to assure their continuous genetic integrity and histocompatibility with other sublines maintained in counterpart genetic production centers.

OHIO STATE UNIVERSITY (N01-CM0-7442)

This is a Phase I evaluation of the pyron copolymer MVE2. Preliminary results are being produced. The study should be completed in 1981.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM6-7060)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is being phased out during this fiscal year.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM8-7161)

The purpose of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. A reverse-phase ion-pair HPLC assay was developed for dihydroanthracenedione (NSC-279836; DHAD) using anthracenedione (NSC-287513; AD), the 1,4-dideoxyanalog of DHAD as the internal standard, and detection at 605 nm. DHAD was found to be unstable in patient samples and an extraction procedure and storage conditions were established to prevent drug degradation. The DHAD assay was evaluated in dogs prior to use in patient studies; thus a complete pharmacokinetic analysis of DHAD in beagle dogs was performed for comparison with the behavior of the drug in humans. DHAD is eliminated by an apparent first-order process in man. There is a rapid drop in plasma concentration following iv bolus administration (the concentration at 200 minutes is approximately 5 to <1% of the initially observed plasma concen-

tration). Using a three-compartment model, $t_{1/2\alpha} = 3.5$ min, $t_{1/2\beta} = 11$ min, and the terminal half-life is approximately 2 hours (range of 0.85 to 5.8 hours). Renal clearance is a minor pathway of elimination representing an average of 2.6% of the corresponding total body clearance values. DHAD is highly bound (>97%) to plasma proteins. No detectable levels were found in three CSF samples obtained 1.5-4 hours following a 12 mg/m^2 iv bolus dose to a patient. An HPLC assay was developed for quantitating aclacinomycin A (NSC-208734; ACM) and several of its metabolites. ACM is eliminated by an apparent first-order process of a three-compartment open model in man. The initial disposition phases are short ($t_{1/2\alpha} = 2.50$ min and $t_{1/2\beta} = 20.1$ min). The terminal half-life is also short ($t_{1/2} = 2.60$ hr). A large apparent volume of distribution of the central and peripheral compartments indicates that ACM is highly bound to tissues. Several metabolites of ACM have been identified and their quantitation in patient samples is under study.

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (N01-CM9-7264)

The purpose of this contract is to study the pharmacological properties of new antitumor agents. Where indicated, pilot studies are first carried out in experimental animals (rats, mice or dogs). Information is then obtained on the absorption, plasma clearance, distribution, plasma protein binding, metabolism, and urinary and biliary excretion of such agents in human subjects. Comprehensive pharmacokinetic studies with potential predictive value are stressed.

ONTARIO CANCER INSTITUTE (N01-CM0-7418)

A member of the Lung Cancer Study Group (LCSG): This is a cohort of five (5) clinical centers supported by a central pathology and statistical center, engaged in the study of potentially resectable non-oat cell lung cancer. There are several protocol studies all actively accruing patients. The first study initiated by the Group evaluated the use of intrapleural BCG + INH given for 12 weeks as adjuvant therapy in resectable Stage I disease. This study has currently completed patient accrual, and has been supplemented by a natural history registry, with new surgical and chemopreventive trials to start patient accrual in 7/81. Stage II and III adenocarcinoma and large cell carcinoma are randomized to a three-drug combination of cyclophosphamide, adriamycin, and cis-platinum versus intrapleural BCG and levamisole. In Stage II and III bronchogenic carcinoma, the role of postoperative radiotherapy is assessed against a control arm. The CAP regimen (cyclophosphamide, adriamycin, cis-platinum) is being evaluated in completely resected advanced Stage I disease and in partially resected locally advanced non-small cell cancer. New protocols proposed for activation by the group include lobectomy versus limited pulmonary resections for Stage I tumors and preoperative chemotherapy and radiation therapy in Stage III squamous cell cancer.

Publications stemming from preliminary work of the LCSG have been accepted in four major journals.

ONTARIO CANCER INSTITUTE (N01-CM9-7267)

This is one of five collaborating institutions evaluating parenteral nutrition as an adjunct to intensive combination chemotherapy in patients with limited or extensive small cell carcinoma of the lung. Patients who are randomized to the nutrition intervention group receive graded levels of nutritional support based on their nutritional status.

PAPANICOLAOU CANCER RESEARCH INSTITUTE (N01-CM8-7230)

This contract provides for a complete pathological, parasitical, and microbiological work-up of breeding stock primarily sent from barrier room expansion colonies and the nude mouse production colonies. This contract expired June 26, 1981. This effort is being recompeted with new award expected June 27, 1981.

PENNSYLVANIA, UNIVERSITY OF (N01-CP8-5654)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contractor is evaluating whole body impedance testing as a measure of body water. Body water is proportionate to fat-free body mass. This contract is being phased out.

PENNSYLVANIA, UNIVERSITY OF (N01-CP8-5658)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contract supports studies of several isotopes as measures of body compartments in cancer patients before and during anti-cancer treatment.

PHARM-ECO LABORATORIES, INC. (N01-CM1-7487)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 60% of the effort of this contract is devoted to the preparation of large quantities of material, in the multikilogram range.

PHILIPS ROXANE LABORATORIES, INC. (N01-CM6-7053)

This resource contract provides the DCT with facilities for development, formulation and production of oral dosage forms of investigational drugs. The dosage forms are manufactured in conformity to FDA Current Good Manufacturing Practices. These dosage forms are packaged, labeled and shipped to the NCI for subsequent distribution to clinical investigators.

PITTSBURGH, UNIVERSITY OF (N01-CB2-3876)

This contract is designed to support the study of adjuvant treatment in breast cancer. Currently, protocols B-04, B-05, B-07, B-08, and B-09 are closed to patient accrual, although all patients are still being followed. Protocol B-06 (segmental mastectomy) is still open for patient accrual. New protocols for Stage II breast cancer testing the role of adjuvant adriamycin, and for Stage I breast cancer have recently been activated.

PITTSBURGH, UNIVERSITY OF (N01-CM7-7177)

This contract is designed to support randomized, controlled studies using adjuvant therapy in treatment of colo-rectal cancer. Present studies include C-01 which randomizes patients having stage B+C colon cancer between no treatment, chemotherapy, and immunotherapy and R-01 which randomizes patients having stage B+C rectal cancer between no treatment, radiotherapy and chemotherapy.

POLYSCIENCES, INC. (N01-CM0-7300)

This service preparative contract provides for the large-scale extraction of various plants and isolation and purification of the active materials for preclinical development and clinical trials. Cost and yield data are obtained on these processes. In the case of those plant processes which will be used again, process development optimization studies are conducted.

POMONA COLLEGE (N01-CM6-7062)

This contract conducted quantitative structure-activity studies on congeneric series of antitumor agents. In addition, the contract provided experimental determination of necessary physicochemical parameters (log P, E_s , sigma) when these values were not available in the literature. This contract terminated in March, 1981.

PURDUE RESEARCH FOUNDATION (N01-CM9-7296)

This contract provides for the fractionation of confirmed active plant extracts in an attempt to isolate in a pure state and identify the active compound(s). Plant material used in this work is obtained, for the most part, through the United States Department of Agriculture.

RALTECH SCIENTIFIC SERVICES, INC. (N01-CM8-7182)

This is a service contract to prepare extracts of plant materials for screening by the screening laboratories. The majority of plant extracts screened by NCI originate with this contract. The majority of the contractor's plants are obtained through a Transfer of Funds Agreement between the NCI and the United States Department of Agriculture. Special studies on methodology of plant extraction are conducted in order to maximize the yield of confirmed active plants.

RESEARCH TRIANGLE INSTITUTE (NCI-CMO-7352)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

RESEARCH TRIANGLE INSTITUTE (N01-CM9-7261)

This procurement contract collects plants in the United States. The plants are then subjected to aqueous extraction and the samples lyophilized and sent to Raltech Scientific Services for testing. Actives will be submitted to the NCI fractionation contractors. This contract was terminated this fiscal year due to budgetary restrictions.

RESEARCH TRIANGLE INSTITUTE (N01-CM9-7313)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources. All materials, whether prepared at the Institute or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by NCI staff.

SASCO, INC. (N01-CM9-0164)

This procurement contract provides for the supply of 234,000 CD2F1 (BABL/c female x DBA/2 male) hybrid mice for Developmental Therapeutics Program compound evaluation studies. Breeding animals originate in genetic centers. Fixed-price contract # 263-81-C-0096.

SIDNEY FARBER CANCER INSTITUTE (N01-CMO-7433)

This is a Phase I and II evaluation of human leukocyte interferon. The IND is being filed with the FDA, and it is estimated that patients will be entered in the study during May, 1981.

SIDNEY FARBER CANCER INSTITUTE (N01-CM4-3781)

This contract is designed to explore in prospective, randomized, controlled protocol studies (1) the value of adjuvant chemotherapy after curative gastric surgery; (2) Phase II studies in advanced gastric cancer; and (3) randomized Phase II studies of combinations in advanced disease. This contract is currently in phase out status.

SIDNEY FARBER CANCER INSTITUTE (N01-CM5-7035)

This contract is designed to support prospective, randomized, controlled protocol studies using combined modalities in the surgical adjuvant treatment of colon and rectal carcinoma. This contract is currently in phase out status.

SIDNEY FARBER CANCER INSTITUTE (N01-CM6-7037)

This contractor conducts Phase II/III studies in patients with solid disseminated tumors. The tumors to be included are limited to lung, breast, melanoma, soft tissue, and bone sarcoma. This contractor will be phased out this fiscal year.

SIDNEY FARBER CANCER INSTITUTE (N01-CM9-7276)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the DCT or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

SIMONSEN LABORATORIES (N01-CM5-0578)

This contract furnishes approximately 234,000 six-week old B6D2F1 (C57BL/6 female x DBA/2 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production centers. Fixed-price contract # 263-81-C-0099.

SIMONSEN LABORATORIES (N01-CM7-7166)

The contractor maintains a primary genetic center of inbred strains of rodents. Small quantities of animals from the colony are made available for tumor transplantation and the majority are furnished for large-scale production colonies from pedigreed expansion colonies. All pedigreed foundation colonies are maintained in associated flora isolators.

SIMONSEN LABORATORIES (N01-CM9-7247)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations. This contract furnishes breeding animals for large-scale production colonies. The breeding stock is received from the primary genetic centers.

SISA, INC. (N01-CM0-7354)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CB5-3815)

This contract was recently transferred to the Biological Evaluation Branch, CTEP, from DCBD. The purpose is to evaluate the adjuvant effect of levamisole in head and neck cancer. The study is in its 5th year of funding. It will be completed within the next year.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CB5-3970)

This contract was transferred to the Cancer Therapy Evaluation Program from DCBD. It consists of the evaluation of immunotherapeutic agents for acute and chronic toxicity, and their effects on the immune system. Agents included are: intravenous BCG, POLY IC-LC, and endotoxin. The study is in its 5th year and results should be available within one year.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CB7-4145)

The objective of this contract is to determine whether the cells of renal cancers have cross-reacting tumor-associated antigens. The contractor will attempt to analyze the specificity of the serological reactions and to investigate the patterns of antigen expression in terms of culture time, growth characteristics, and cell cycle kinetics. Periodic assessment of tumor immunity will be performed on sera from patients with Stage I renal cancer receiving conventional therapy and on sera from patients with Stage II, III, and IV renal cancer who are immunized with BCG and allogeneic renal cancer cells.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CB7-4146)

Iravesical and percutaneous BCG are being evaluated for their efficacy in patients with recurrent superficial bladder cancer. Preliminary results suggest an advantage for BCG treatment. Continuation of this study is planned.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CM0-7435)

This contract is for Phase I and II clinical trials utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in May, 1981, at which time patient accrual will start.

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (N01-CM0-7463)

This contract supports a Phase I study of the agent AZQ in pediatric patients. This study is expected to enroll 25-30 patients at various doses to determine the toxicity and pharmacology of the agent. Possible age-related differences in effects shall also be taken into account. Pharmacological studies will evaluate the blood kinetics by appropriate measurement in vitro.

SMALL BUSINESS ADMINISTRATION (N01-CM4-3719)

The purpose of this contract is to provide supportive services in small animal studies, immunology, and tissue culture. At the present time, these functions include primarily the following: (1) detailed karyotypic analysis, including Giemsa banding, of a variety of monolayer and suspension cultured cells; (2) evaluation of tumorigenicity of various cultured cells by inoculation into nude mice; (3) testing the tumorigenic potential of selected primate retroviruses; (4) preparing small quantities of selected (frequently cloned) cells and retroviruses; and (5) testing various tissue cultured cell specimens for mycoplasma contamination.

SOUTH FLORIDA, UNIVERSITY OF (N01-CM8-7220)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

SOUTHERN ANIMAL FARMS (N01-CM5-0599)

This contract furnishes approximately 234,000 hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. Fixed-price contract # 263-81-C-0101.

SOUTHERN ANIMAL FARMS (N01-CM9-7245)

This contract provides for the maintenance of a rodent production center. This center produces stocks and strains of rodents for research and testing laboratory programs. Production levels for individual colonies are correlated with requirements for specific investigations.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CM5-3842)

This contract is designed to explore in prospective, randomized, controlled protocol studies (1) the value of adjuvant chemotherapy following a curative gastric resection, and (2) Phase III evaluation of single agent and combination chemotherapy regimens in advanced disease. The contractor is also performing Phase II single agent studies in patients who have previously received chemotherapy.

SOUTHERN RESEARCH INSTITUTE (N01-CM0-7260)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

SOUTHERN RESEARCH INSTITUTE (N01-CM8-7162)

The purpose of this contract is to obtain basic quantitative pharmacologic information in animals which will indicate the requirements of drug dosage and scheduling necessary to give maximum tumor cell kill while minimizing toxicity to the host's normal cells. This involves the development and application of highly sensitive analytical methodology to measure drugs and their metabolites in body fluids and tissues and the estimation of half-lives and cumulative Cxt values in animals and man.

SOUTHERN RESEARCH INSTITUTE (N01-CM9-7263)

The objective of this contract is to explore the pharmacologic and toxicologic effects of antitumor drugs administered singly and in combination with other modalities to animals. These other agents may be another antitumor drug, an inhibitor of metabolism of the active antitumor agent, compounds that affect the immune system or a physical agent, for example x-irradiation.

SOUTHERN RESEARCH INSTITUTE (N01-CM9-7309)

This project is designed to provide to DCT a maximally flexible single instrument for the rapid conduct of Program-directed developmental and applied studies pertinent to all preclinical, therapy-related, tasks. Principal tasks are: (1) to apply fundamental biological principles to the development of new and improved laboratory models for the discovery of more effective antitumor drugs; (2) to evaluate, in detail, drugs in development to NCI sponsored clinical trial to determine their optimum conditions of usage; (3) to conduct in vivo screening against transplantable animal tumors and human tumor xenografts; and (4) to conduct studies leading to the refinement of screening protocols.

SOUTHWEST FOUNDATION (N01-CM0-7356)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

SRI INTERNATIONAL (N01-CM0-7351)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

SRI INTERNATIONAL (N01-CM8-7183)

SRI International is the major contractor responsible for the chemical analysis of bulk chemicals and clinical formulations which are to be investigated in the pharmacological, toxicological and clinical phases of the Developmental Therapeutics Program. The contractor determines the identity and purity of the compounds by appropriate methods. Also solubility, stability and other physical-chemical properties are determined to provide information as a guidance to the effective use, formulation and storage of the materials. Techniques commonly used include elemental analysis, chromatography (paper, thin-layer, gas liquid

SRI INTERNATIONAL (N01-CM8-7183) (CONTINUED)

and high-pressure liquid), spectroscopy (ultraviolet, infrared and nuclear magnetic resonance), gas chromatography/mass spectrometry and other methods as needed. Reports of the analytical testing provide data to be included in IND filings with the FDA and for quality assurance monitoring. This is the larger of the two contracts for providing this service.

SRI INTERNATIONAL (N01-CM8-7207)

The objective of this cooperative effort between SRI International and Stanford University is the synthesis and biological evaluation of nitroimidazoles and other related nitroheterocycles. Compounds are selected for further development based on determinations of electron reduction potential, lipophilicity, peak drug levels in both plasma and tumors, and neurotoxicity.

SRI INTERNATIONAL (N01-CM9-7256)

This service preparative contract provides for the synthesis of radiolabeled chemicals and drugs for use in preclinical pharmacologic and clinical studies. Many of the materials prepared are not available from commercial sources and are synthesized. All materials, whether prepared by the contract group or acquired from other sources, are analyzed for purity and identity by radioautography assay, etc. This contract also provides storage facilities for labeled materials and distributes such as directed by NCI staff.

ST. LOUIS UNIVERSITY SCHOOL OF MEDICINE (N01-CM5-7020)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated. This contract is being phased out during this fiscal year.

STARKS ASSOCIATES, INC. (N01-CM0-7357)

The newly initiated Quick Reaction Work Order Contract mechanism provides for the synthesis of a variety of organic and/or inorganic compounds which have been identified by Program as meriting development. The Quick Reaction Work Order contracts provide the capability to pursue leads uncovered by biological testing, by the resynthesis of compounds in quantities sufficient for confirmatory testing. Another objective is the synthesis of a selected number of novel analogs for structure-activity optimization studies. The Task Order contract makes available several contractors who have the chemical synthetic expertise to synthesize a variety of compounds. The mechanism provides the capability of a quick response, makes accessible a pool of contractors, isolates cost for each task, provides flexibility for funds, and avoids prolonged contract competitions.

STARKS ASSOCIATES, INC. (N01-CM1-7374)

This service preparative contract is for the resynthesis of bulk chemicals and drugs required for completion of drug evaluation studies, with approximately 50% of the effort being devoted to the production of clinical materials. The materials assigned for resynthesis are not readily available in the quantities needed from the original supplier or on the open market. Preparations vary in quantity from gram to multikilogram scale.

STARKS ASSOCIATES, INC. (N01-CM8-7206)

This contract is in support of the Drug Synthesis and Chemistry Branch's (DS&CB), DTP, fundamental responsibility to acquire selected novel synthetic compounds for evaluation as potential anticancer agents, the initial step in the NCI's Linear Array for Drug Development. The major focus of this contract is the active solicitation, acquisition and management of the flow of approximately 13,000 compounds per year of diverse structural types. These compounds are selected by the DS&CB from a much larger pool of compounds provided through this contract in quantities adequate for the primary anticancer screen. This contract also acquires a significant proportion of the larger samples needed for secondary screening (Tumor Panel) of the new leads that are identified.

TACONIC FARMS (N01-CM5-0597)

This contract furnishes approximately 160,000 B6D2F1 (C57BL/6 female x DBA/8 male) hybrid mice for compound evaluation studies. Breeding animals are furnished from genetic centers and/or rodent production colonies. This contract expired March 13, 1981.

TENNESSEE, UNIVERSITY OF (N01-CM1-7472)

The clinical data from patients with intracranial malignancy provided under this contract are used to evaluate the efficacy of therapy in both Phase II and Phase III clinical trials. Multimodality therapy, including surgery, radiation therapy, chemotherapy, and various sequences and combinations thereof, is used

TENNESSEE, UNIVERSITY OF (N01-CM1-7472) (CONTINUED)

according to a commonly agreed upon protocol in patients who harbor histopathologically diagnosed brain tumors. Single modality therapy is first demonstrated to have efficacy and then forwarded into combined modality treatment protocols. New chemotherapeutic agents, as they are developed and brought to clinical trial, are also being tested in controlled Phase II trials in order to provide a more rational selection of chemotherapeutic agents for further Phase III studies of brain tumor patients. The acquisition of a significant number of patients into these prospective, controlled, randomized studies permits a comparative analysis of the clinical course of the patients, the toxicity of the various therapeutic modalities utilized, as well as insight into the biologic characteristics of the disease entity being investigated.

TEXAS INSTITUTE FOR REHABILITATION AND RESEARCH (N01-CP8-5618)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contract supports studies of stereometric photography as a measure of body surface area, body volume, and body density. From body density, body fat and muscle may be estimated.

TEXAS, UNIVERSITY OF (N01-CB8-4248)

The objective of this contract is to evaluate, in a controlled clinical study, the effect of intralesional injection of BCG crude cell walls on canine breast carcinoma. Dogs clinically free of detectable metastatic disease are randomly assigned to intralesional immunotherapy prior to surgery. The BCG crude cell walls are provided by the NCI. Tumor regression, tumor recurrence, disease-free interval, and survival data from the two are compared. Selected assays of humoral and cellular immunity are performed and results correlated with clinical course. Approximately 100 dogs per year have been entered in the study. This contract is due to expire in September, 1981.

TEXAS, UNIVERSITY OF (N01-CM0-7417) (FORMERLY N01-CM7-7153)

Lung Cancer Study Group Pathology Reference Center: A special task contract to review cytology, surgical biopsies, and autopsy material provided by the Lung Cancer Study Group.

TEXAS, UNIVERSITY OF (N01-CM5-3832)

This contract assessed a quantitative evaluation of the protected environment for hypoplastic individuals and great risk for infection. This program was phased out during this fiscal year.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (N01-A10-2659)

The Biological Response Modifiers Program funded this project entitled "Antisera to Immune Interferons" in Fiscal Year 1980. The contract is administered by NIAID and expires on August 31, 1982. The objective of the contract is to produce antisera to human immune (type II) and mouse immune (type II) interferons in the required quantity to be used as NIH reference agents.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (N01-CM8-7221)

A member of the Head and Neck Contracts Program: A collaborative group of eight (8) institutions and two (2) cooperative oncology groups to investigate the effects of adjuvant chemotherapy in the treatment of advanced, resectable squamous carcinoma of the head and neck. The contract study is a prospective, randomized comparison of a standard treatment regimen consisting of surgery and postoperative radiotherapy with a regimen consisting of induction chemotherapy followed by the standard regimen, and with induction chemotherapy followed by the standard regimen with the addition of a six (6) month course of maintenance chemotherapy. The induction chemotherapy consists of cis-platinum and bleomycin. Cis-platinum alone is utilized as the maintenance chemotherapy. Two-hundred and three (203) patients have been randomized to the study during the first 17 months since its activation.

TEXAS, UNIVERSITY OF, M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (N01-CB3-3888)

The purpose of this contract is to develop immunologic agents for cancer treatment. Included are Phase I and II studies with MER, C-Parvum, BCG, and thymosin. Linear followup of treated patients is continuing. This contract is scheduled to terminate in January, 1982.

TEXAS, UNIVERSITY, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM0-7342)

This contract supports a Phase I study of the agent DON in pediatric patients. This study is expected to enroll 25-30 patients at various doses to determine the toxicity and pharmacology of the agent. Possible age-related differences in effects shall also be taken into account. Pharmacological studies will evaluate the blood kinetics by appropriate measurement in vitro.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM0-7406)

This contract conducts Phase II/III studies in patients with solid disseminated tumors. The tumors included are of the lung, breast, prostate, bladder, kidney, testicle, ovary, endometrium, cervix, head and neck, stomach, pancreas, and colon, as well as lymphomas, melanomas, and bone and soft tissue sarcomas. A minimum of 200 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy, or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM6-7026)

Ovarian Cancer Study Group Pathology Reference Center: A special task contract to review surgical and postmortem specimens submitted by the Ovarian Cancer Study Group. This contract is currently in phase out status.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM6-7116)

A collaborative group whose main thrust is to study various post-surgical adjuvants in early epithelial tumors of the ovary. A joint protocol for good risk Stage I (i.e., FIGO Stage IA1 and IB1) compares observation to L-PAM post-operatively. A secondary study involves a histopathologic study of the incidence of unsuspected pelvic and/or paraortic node metastases in this patients population. A further joint protocol for other early disease (i.e., FIGO Stage IA1i, IB1i, IC, and II) separates patients according to presence or absence of macroscopic residual disease postoperatively. Patients with macroscopic residual disease are randomized to L-PAM versus L-PAM + pelvic radiotherapy. Patients without macroscopic residual disease are randomized to L-PAM versus intraperitoneal instillation of 15 mc of radioactive chromic phosphate. This contract is currently in phase out status.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM8-7185)

The purpose of this contract is to carry out studies on the pharmacology and physiological disposition of new antitumor agents under development by the NCI. During the current year, studies have been completed on AT-125, AZQ, dihydroanthracenedione, 5-methyltetrahydrohomofolate, methylglyoxal-bis(guanylhydrazone) and vindesine. Studies under this contract are carried out in experimental animals and in cell culture lines, but are closely interrelated with Phase I clinical trials conducted at the M.D. Anderson Hospital and Tumor Institute and elsewhere.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM8-7233)

The purpose of this contract is to collect pharmacokinetic data on new and established antitumor agents in patients undergoing treatment for malignant disease and to analyze these data for individual variability which can be correlated with clinical response or some other pharmacologic parameter. Continuous infusion adriamycin was studied in a large group of patients because of the reports that higher total doses of adriamycin can be given by this procedure without causing cardiac damage. Pharmacokinetic and toxicologic parameters were determined in patients infused for 48 to 96 hours at doses from 960 - 1260 mg/m². Certain metabolites of adriamycin were also quantitated. An HPLC analysis was developed for pentamethylmelamine and its demethylated metabolites. Both parent pentamethylmelamine and cytotoxic demethylated products were quantitated in patient samples as a function of dose and time after dose administration. This contract was terminated during this fiscal year.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CM9-7277)

This contract is designed to conduct Phase I and Phase II studies with new anti-cancer drugs sponsored by the DCT. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (N01-CP6-5655)

This is one of eight contracts on the subject of nutritional assessment which is funded by the Diet, Nutrition, and Cancer Program, OD, and administered by DCT. This contract supports studies of assessment of the nutritional status of pediatric cancer patients. In addition to anthropometric measurements, lymphocyte function is being measured as a monitor of target cell effects of under-nutrition. This contract has been phased out.

UPJOHN COMPANY (N01-CM0-7380)

This contract has as its primary objective the development of potentially useful antineoplastic agents from fungi, bacteria and actinomycetes, fermentation beers, including isolation, purification, characterization and production.

The Upjohn Company has, for many years, played a leading role in the development of cell culture methods for antitumor drug development. The methods have been used for assays on fractionation samples and for primary screening. This company used many in vitro prescreens. Leads developed from these screens are being tested in-house in the leukemia in vivo screen and actives are given top priority for chemical fractionation.

VANDERBILT UNIVERSITY MEDICAL CENTER (N01-CM0-7438)

This is a Phase I evaluation of the pyron copolymer MVE2. Preliminary results are being produced. The study should be completed in 1981.

VERMONT, UNIVERSITY OF (N01-CM9-7278)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the DCT or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

VETERANS ADMINISTRATION (Y01-CM7-0106)

This is an Interagency Agreement with the Veterans Administration Hospital to conduct the Meical Oncology Branch whose primary objective is to study lung cancer and other adult tumors in the Veterans Administration Hospital. This contract was originally set up as a means of expanding our intramural capability

VETERANS ADMINISTRATION (Y01-CM7-0106) (CONTINUED)

to study common human tumors. Lung cancer, being the number one cancer killer in men, was selected as a prime target for clinical trials with cancer chemotherapy. Clinical trials and related laboratory investigations have been designed in a variety of tumors seen commonly at the Veterans Hospital. The Veterans Hospital provides a resource of patient material for studies which could not be undertaken at the Clinical Center. This medical oncology resource allows an expansion of our intramural drug-testing effort. This unit will be transferred to the National Naval Medical Center during Fiscal Year 1982.

VETERANS ADMINISTRATION (Y01-CM7-0107)

The VA Surgical Oncology Group is supported under this Interagency Agreement. This Group, which has been in existence for some 23 years, conducts trials evaluating chemotherapy, radiotherapy, and/or immunotherapy as adjuncts to surgical treatment of lung, gastric, pancreatic, esophageal, rectal, colon, and head and neck carcinomas. Ancillary studies include the identification of prognostic factors, the significance and evaluation of hormonal markers and CEA, and histopathologic classification. This Agreement is being terminated with phase out funding to September, 1982. All studies have been closed and final manuscripts are now being prepared.

VSE CORPORATION (N01-CM0-7251)

This contract was awarded as the result of a competition held in calendar year 1979. Data processing services are provided to the Developmental Therapeutics Program by this contract. The scope of work includes (1) reducing and disseminating information developed in the screening program of the Drug Evaluation Branch to both staff and the suppliers of the compounds being tested; (2) documenting all computer programs and contractor's procedures for data handling and running computer programs; (3) maintaining computer programs so that they are able to run in the Division of Computer Research and Technology environment; (4) modifying the existing data system so that data from new antitumor systems can be handled (e.g., the Human Tumor Stem Cell Cloning Assay, and the Astrocytoma In Vitro Assay); (5) refining the data collection methods; (6) providing instructions for screening laboratories and suppliers of materials relating to collection and dissemination of data; (7) providing output for statistical evaluation of test systems and evaluation of test system parameters; and (8) participation in scientific meetings.

VSE CORPORATION (N01-CM8-7192)

The Drug Distribution and Protocol Monitoring System (DDPMS) is an automated procedure for verifying the accuracy of requests made by clinical investigators for investigational and commercial anticancer drugs. This verification is mandated by the FDA since federal law requires that drugs used for investigational purposes only be given in sufficient quantity to authorized users for approved protocols. Verified data are retained and form a drug distribution history which is used to monitor protocol progress as clinical trials progress.

VSE CORPORATION (N01-CM8-7192) (CONTINUED)

This protocol monitoring function is also an FDA requirement imposed on the NCI. The DDPMS is composed of numerous computerized files containing investigators names and addresses, the protocol information file (investigator name, number, approved protocols, drug dosage form, registration record), the IND Drug Activity File (drug distribution history by protocol) and the patient report file. In addition, the system has reading capability to retrieve inventory and drug cost data from a system operated by the Pharmaceutical Resources Branch, DTP.

WARNER-LAMBERT COMPANY (N01-CM1-7491)

This service preparative contract provides for the resynthesis of a variety of compounds required for clinical or preclinical evaluation. The compounds prepared are not readily available on the open market or from the original supplier in the amounts required. About 30% of the effort on this contract is devoted to the preparation of large quantities of materials, in the multikilogram range, requiring pilot plant facilities.

WARNER-LAMBERT COMPANY (N01-CM0-7292)

This contract is to develop a protocol method of producing 50 billion units of leukocyte interferon with a purity of at least 3×10^6 units/mg protein. Fifty billion units, meeting all GMP and Bureau of Biologics standards, will be produced in the two years of this contract.

WARNER-LAMBERT COMPANY (N01-CM0-7379)

This fermentation contract is designed primarily to obtain novel antitumor agents. This contract includes: (1) the preparation of fermentation beers from various microbes isolated from unique substrates from various parts of the world and fermented under a bevy of environmental and stress situations; (2) an in vitro tissue culture assay laboratory which assists in prescreening fermentation broths for cytotoxicity and is used to help assay chemical fractions, fermentation improvement samples and large pilot plant batches more quickly; (3) a small biotransformation program; (4) the isolation work required to obtain the active component from the confirmed active beers; (5) the production of large quantities of antineoplastic agents approved for clinical trials; and (6) the preparation of appropriate dosage forms of such agents.

WASHINGTON, UNIVERSITY OF (N01-CB8-4247)

The objective of this contract is to determine the conditions and operative mechanisms for achieving optimal therapeutic effect of adoptive cellular immunotherapy used alone or in combination with chemotherapy in the treatment of tumors of C57BL/6, BALB/c and CB6F1 (BALB/c female x C57BL/6 male) mice. The approach is to utilize, in adoptive cellular immunotherapy experiments, cells immunized both in vitro and in vivo. Studies will be undertaken to characterize the effector cell(s) responsible for tumor therapy as well as cells which suppress

WASHINGTON, UNIVERSITY OF (N01-CB8-4247) (CONTINUED)

the therapeutic effect. The role of the antigens for the major histocompatibility complex in sensitization to tumor associated antigens and in the generation of cells therapeutically effective against established syngeneic tumors will be explored. This contract is due to expire in September, 1981

WASHINGTON, UNIVERSITY OF (N01-CM9-7282)

This contract provides a cyclotron neutron generator, a clinical facility in which to house the equipment, and personnel to support a clinical neutron therapy research program at the University of Washington. The subcontract for the fabrication of the cyclotron was awarded to Scanditronix/Nucleotronix and the A/E subcontract for the building addition was awarded in May, 1980. The facility should be operational in early 1983.

WAYNE STATE UNIVERSITY (N01-CM0-7404)

The contractor conducts Phase II/III studies in patients with solid disseminated tumors. A minimum of 400 patients a year is studied, with no less than 25 patients in any tumor type. These patients are treated intensively with chemotherapy either alone or in combination with radiotherapy, immunotherapy or surgery in protocols agreed upon by the Project Officer and Principal Investigator.

WAYNE STATE UNIVERSITY (N01-CM0-7408)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

WAYNE STATE UNIVERSITY (N01-CM9-7279)

This contract is designed to conduct Phase I and Phase II studies with new anti-cancer drugs sponsored by the DCT. The Phase II studies will be conducted in the DCT panel tumors: acute leukemia, lymphoma, melanoma, and carcinoma of the lung, breast, and colon.

WELLCOME FOUNDATION (N01-CM1-7489)

This contract was for delivery of 25 billion units of lymphoblastoid interferon, 3×10^7 units/mg protein, and has been successfully completed.

WISCONSIN, UNIVERSITY OF (N01-CM0-7434)

This contract is for Phase I and II clinical trials utilizing human fibroblast interferon. It is estimated that the interferon for these trials will be available in May, 1981, at which time patient accrual will start.

WISCONSIN, UNIVERSITY OF (N01-CM9-7280)

This contract is designed to conduct Phase I clinical studies with new anticancer drugs sponsored by the DCT or to conduct Phase I clinical studies with new combinations or regimens mutually agreed upon.

WISCONSIN, UNIVERSITY OF (N01-CM9-7289)

This contract provided cytotoxicity screening of crude natural plant, animal, and microbiological products for potential antitumor activity. Isolation and purification of active crude materials were also followed by means of these in vitro assays. A significant effort was also expended on development of a modified assay utilizing less material and less time. This contract was phased out during this fiscal year and terminated on February 28, 1981.

WISCONSIN MEDICAL COLLEGE (N01-A10-2658)

The Biological Response Modifiers Program funded this project entitled "Immune Interferon Standards" in Fiscal Year 1980. The contract is administered by NIAID and expires on August 31, 1982. The objective of the contract is to provide NIH with 1,200 ampoules of (1) a suitably stable, potent, freeze-dried immune interferon prepred from human lymphocytes and (2) an equal number of a similarly suitable preparation prepared from mouse lymphocytes for use as standard reference reagents.

YALE UNIVERSITY (N01-CB7-4191)

This contract has recently been transferred from DCBD. It consists of the evaluation of intratumor-given BCG prior to surgical resection in patients with lung cancer. The trial is in its 5th year. It is expected to be completed in one year.

YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CM0-7339)

This contract is designed to explore in prospective, randomized, controlled protocol studies: (1) the value of adjuvant chemotherapy following a curative gastric resection; (2) the value of adjuvant chemotherapy plus radiotherapy for a curative pancreatic resection; (3) the value of combined radiotherapy and chemotherapy for locally unresectable pancreatic and gastric cancer; and (4) the value of various drug combinations or new agents in advanced gastric and pancreatic cancer.

YAMANOUCHI PHARMACEUTICAL CO., LTD. (N01-CM9-7307)

The objectives of this contract are to provide facilities and capabilities for the development and production of parenteral investigational dosage forms for the DCT. The contractor is responsible for conformity to U.S. FDA Current Good Manufacturing Practices and for completing all required analytical testing on each product prepared. In addition, the contractor is responsible for stability surveillance on all dosage forms produced. All products are packaged, labeled and shipped to the NCI for subsequent redistribution to clinical investigators.

REPORT OF THE ASSISTANT DIRECTOR FOR INTERNATIONAL TREATMENT RESEARCH

OFFICE OF THE DIRECTOR

The international programs in which the Division of Cancer Treatment is engaged continued to make progress during the year. The various aspects are coordinated by the Office of the Assistant Director for International Treatment Research which was initiated in 1976 within the Office of the Director, Division of Cancer Treatment. Included in the office are: A. Goldin, Assistant Director for International Treatment Research, O. Yoder, NCI Collaborative Office representative, Brussels, Ethel Meyer, Secretary, and Marlana Gelboin, Part-time Clerk-Typist.

The office for International Treatment Research fulfills an important coordination function in which the program interests of the Division of Cancer Treatment are related to international treatment research. Included in these activities are the development of collaborative clinical and preclinical programs, the monitoring of international developments in treatment, personnel exchange, information exchange, identification and procurement of synthetic agents and natural products for the screening and development program and the identification and procurement of new compounds from abroad that are of potential clinical interest. The activities of the Office of the Assistant Director for International Treatment Research is coordinated closely with the Office of the Associate Director for International Affairs, NCI, and close liaison is maintained with important international organizations including the European Organization for Research and Treatment of Cancer (EORTC), the International Union Against Cancer (UICC), the World Health Organization (WHO), the Pan American Health Organization (PAHO) and the International Agency for Research on Cancer (IARC).

The Cancer Chemotherapy Research Collaborative Office under the leadership of the Principal Investigator, Prof. H. J. Tagnon, has been in operation at the Institut Jules Bordet, Brussels, Belgium, since its establishment in 1972. It provides a highly important service function for the international program of the Division of Cancer Treatment. It represents a center of reference which makes available to research workers and cancer specialists in the U.S. and Europe all of the pertinent information on the ongoing cancer research programs on both continents.

The Collaborative Office in Brussels is well known by the scientific community in cancer research in Europe and is accepted as the intermediary link between the two scientific communities, Western Europe and North America. In this regard it is fulfilling an irreplaceable function which is that of bringing together leading cancer investigators of the two continents and creating the contacts for exchange of ideas and information. The office is also fulfilling the important function of an observation center where European scientific news and events are gathered and communicated to all interested parties in the U.S.A. Conversely, this has now become the office to which Europeans turn to obtain first-hand information on scientific activities and developments in the U.S.

The Liaison Office has been highly useful in the areas of experimental and clinical pharmacology, clinical trials, exchange of professional personnel, and in the organization of workshops and symposia conducted jointly with participation of American and European investigators.

The close relationship of the Liaison Office with European Pharmaceutical and Chemical industries has provided a continuous flow of chemical agents for evaluation of potential anticancer activity. Since 1972 more than 25,000 new compounds have been collected through the direct efforts of the Liaison Office and several new compounds have proven of clinical interest and are currently being developed toward clinical trial.

The activities of the Liaison Office are closely associated with that of the EORTC whose headquarters are at the Institut Jules Bordet. Included are the office of the Division of Therapeutic Research, the coordinating center for the EORTC cooperative groups, the Data Center which provides a central repository for data on clinical trials in Europe, and the EORTC Foundation, which provides financial support from European sources to the EORTC.

The Data Center of the EORTC now serves 40 clinical groups engaged in therapeutic research. At the end of the last year it was monitoring 64 clinical studies from 255 institutions in 13 countries in which over 1500 clinicians participated. The Data Center staff, in addition to a Director who is both a physician and a biostatistician, includes 3 statisticians, 3 computer analysts and programmers, 8 data managers and 4 medical secretaries. It is currently studying 6500 patients and 1700 new patients are registered each year.

The Institut Jules Bordet in collaboration with the EORTC and the NCI has been in the process of expanding the drug development program for Phase I and Phase II clinical trials. This is a means whereby the number of new drugs evaluated in the clinic can be increased at no additional cost to the NCI program.

Although the foreign exchange program in oncology nursing was making significant progress, the exchanges in this area have been diminished because of budgetary considerations.

Additional symposia were organized during the year with joint American and European participation. An NCI-EORTC Symposium on Nature, Prevention and Treatment of Clinical Toxicity of Anticancer Agents was held at the Institut Jules Bordet, Brussels, on September 25-26, 1980.

FRANCE

The French-American Agreement for cancer clinical research involving the Division of Cancer Treatment, NCI, and the Institut National de la Sante et de la Recherche Medicale (INSERM), plus the respective affiliated institutions, continued its activities during the year.

The annual joint committee meeting of the Cancer Clinical Research Committee of the French-American Agreement was held at the National Cancer Institute in Bethesda, Maryland, December 1-2, 1980. Five working groups were established including (1) Working Group on Clinical Trials - new drugs. Chairmen Macdonald/Boiron. (2) Working Group on Clinical Biochemical Pharmacology. Chairmen Mihich/Cano(Paoletti to rotate between (1) and (2). (3) Working Group on Biological Response Modifiers. Chairmen Oldham/De Maeyer. (4) Working Group on Radiation Sensitizers - Radiopotentiators and Combined Radiation Modalities. Chairmen Phillips/Tubiana. (5) Working Group on Epidemiology. Chairmen Blot/de The. 19 collaborative projects were approved at the December 1-2 meeting and allocated to the relevant working groups as follows:

CLINICAL TRIALS - NEW DRUGS

Cooperative Projects Approved (Classification)	French Collaborators	American Collaborators	Duration
1. Preclinical and early clinical studies of new nitrosourea analogs (Old Project/New Support)	Mathe, G. Villejuif	Schein, P. Georgetown Univ. Washington, D.C.	3 years
2. Therapeutic Effects and Mechanism of Action of Ellipticines (New Project)	Paoletti, C. Villejuif	Lehman, IR, Stanford Univ. Grossman, L. Johns Hopkins	2 years
3. Study of Therapeutic and Pharmacokinetic Differences between Semi-Synthetic Derivatives and Original Compounds especially in the field of Anthracyclines and Antifolates (Old Project/New Support)*	Weil, M. Hopital St. Louis Paris	Holland, J., Mount Sinai School of Medicine New York	3 years
*This project is shared with the Clinical Biochemical Pharmacology Working Area.			
4. In Vivo biochemical effects on endometrial cancer of estrogens, antiestrogens and progestagens. (New Project)	Robel, P. Bicêtre	Mortel, R. Milton Hershey Medical Center Pennsylvania	3 years
5. Phase II Protocol for very high risk Leukemias in Childhood. (New Project)	Schaison, G. Hopital St. Louis Paris	Simone, J., Rivera, B. St. Judes Hospital, Memphis	3 years
6. Chemotherapy of Digestive Tract Carcinomas. (Old Project/New Support)	Gisselbrecht, C. Hopital Saint Louis Paris	Schein, P. Georgetown U. Washington, D.C.	3 years
7. Phase II Therapeutic Trials with Chemotherapy and Immunotherapy in Advanced Cancers. (Old Project/New Support)	Israel, L. Bobigny	Schein, P. Georgetown U. Washington, D.C.	3 years
8. Evaluation of Insulin in the Treatment of the Cachectic State of Cancer Patients, Randomized Study. (New Project)	Serrou, B. Centre Paul Lamarque Montpellier	Schein, P. Georgetown U. Washington, D.C.	2 years
9. Methodology for Estimation of the Clinical Efficacy of anticancer Agents. (New Project)	Marty, M. Hopital St. Louis, Paris	Macdonald, J. NCI, Bethesda, Maryland	3 years

CLINICAL BIOCHEMICAL PHARMACOLOGY

Cooperative Projects Approved (Classification)	French Collaborators	American Collaborators	Duration
1. Biochemical, Pharmacological and Clinical (Phase I-II) Studies of New Platinum Coordination Compounds. Application to Citrate Derivatives. Analysis of Pt-DNA Adducts by Mass Spectrometry. (New Project)	Macquet, J.P. CNRS Toulouse	Wiebers, J. Purdue Univ. W. Lafayette Indiana	3 years
2. Pharmacokinetics of Cyclophosphamide and Metabolites. (New Project)	Boiron, M. Hopital St. Louis, Paris	Mihich, E. Roswell Park Memorial Inst. Buffalo, N.Y.	3 years
3. Clinical studies of the pharmacology and metabolism in vitro and in vivo of the Fluoropyrimidines. (Old Project/New Support)	Carcassone, Y. Inst. Paoli- Calmettes Marseille	Rustum, Y. Roswell Park Buffalo, N.Y.	3 years
4. Study of the anti-tumor activity of a new type of drug-carrying liposome coupled to monoclonal antibody. (New Project)	Kourilsky, F. Leserman, L. Marseille	Weinstein, J. Hodes, R. NCI, Bethesda	2 years
5. Pharmacokinetics and metabolism of anthracyclines. (Old Project/New Support)	Lagarde, C. Robert, J. Fondation Bergonie, Bordeaux	Rustum, Y. Roswell Park Memorial Inst. Buffalo, N.Y.	3 years
6. Studies of a new series of antitumor agents: DNA-bis-intercalating Dimers. (New Project)	Roques, B. Faculte de Pharmacie Paris	Tsai Kent State U. Ohio	2 years
7. Modulation of the antileukemic activity of cytosine arabinoside by thymidine. (New Project)	Zittoun, R. Hotel Dieu Paris	Creaven, P.J. Rustum, Y. Roswell Park Buffalo, N.Y.	3 years
8. Liposomes as a delivery system for Anthracyclines. (Old Project)	Mathe, G. Villejuif	Schein, P. Georgetown U. Washington, D.C.	Ongoing

BIOLOGICAL RESPONSE MODIFIERS

<u>Cooperative Projects Approved (Classification)</u>	<u>French Collaborators</u>	<u>American Collaborators</u>	<u>Duration</u>
1. Restoration of Age-Immuno-depressed Mice and Prevention of Spontaneous Tumors with Chemically Defined Immunomodulators. Study of the Mechanisms Involved. (New Project)	Florentin, I. Villejuif	Hadden, J.W. Sloan-Kettering Cancer Center New York, N.Y.	3 years

EPIDEMIOLOGY

<u>Cooperative Projects Approved (Classification)</u>	<u>French Collaborators</u>	<u>American Collaborators</u>	<u>Duration</u>
1. Hepatitis B Virus and Primary Carcinoma of the Liver in Tropical Africa. (New Project)	Coulaud, J.P. Hopital Claude Bernard Paris	Blumberg, B. Inst. for Cancer Res. Philadelphia Pennsylvania	3 years

RADIATION THERAPY

Cooperative Projects Approved (Classification)

None as yet.

Plans were also made at this meeting for scientist interchange, meetings and workshops, and drug exchange.

JAPAN

The Joint Symposium and Business Meeting of the USA-Japan Cooperative Cancer Research Program, Sixth Annual Program Review Meeting of the Treatment Area, was held July 7-9, 1980 in Tokyo.

The USA-Japan Cooperative Cancer Research Program is in its seventh year and the treatment area, which from the beginning has been an important aspect of the Agreement has been making definitive progress. In the first five years the Agreement was organized so that there were 11 different sections, including chemotherapy, radiation, immunology and disease oriented sections, and the treatment aspects of the Agreement were fragmented to some degree within all of these different sections. With the renewal of the Agreement for a second five-year period, the program was reorganized into only four sections, one of which is the treatment area, and all of the aspects of treatment are now incorporated into this part of the Agreement. Thus, treatment is no longer fragmented among the various modalities but is considered as a coordinated interdisciplinary problem, and for the treatment of any one of the various types of cancer, stress is placed on a multidisciplinary strategy.

One of the key aspects of the initial treatment portion of the Agreement pertained to the emphasis on new drugs, including their preclinical and clinical evaluation and development. New drug evaluation and development continues to be an important part of the Agreement and there has been a vigorous exchange of drugs for both preclinical and clinical evaluation between the two countries. Bleomycin and Mitomycin C are established drugs in cancer chemotherapy in the U.S. and drugs such as neocarzinostatin and toyomycin have had clinical evaluation. There is an interest in clinical evaluation of aclacinomycin in the U.S. There is a continuing interaction and flow of drugs from Japan to the USA, enriching the chemotherapy potential of the USA treatment program.

Within the framework of the treatment agreement there are now scheduled three meetings a year. An attempt is being made through the diversity of the meetings to cover all of the major aspects of interdisciplinary interaction on a disease oriented basis, as well as the critical areas of modality development. Within the current year there was a meeting on lung cancer in Osaka in which there was consideration of new developments in the immunotherapy, radiation therapy and chemotherapy of lung cancer and the multidisciplinary approach in lung cancer. Joint Symposia were also held on High LET Radiation and Combined Modalities, and on Treatment of Breast Cancer with Combined Modalities. There is the prospect of continuous and vigorous exchange of scientific activity between the two countries in the coming years.

Indicative of the progress that has been made is the joint trial in the chemotherapy of stomach cancer. This trial involves a joint protocol using common forms, common statistical technique and a common arm of chemotherapy and has demonstrated that not only can the two countries exchange information meaningfully but that they can work together in a clinical research setting. The joint trial constitutes an example of the kind of true collaborative research that is possible between the two countries under the aegis of the Agreement.

The Contract on "Operation of a Collaborative Office for Cancer Chemotherapy Research" under the leadership of Dr. Yoshio Sakurai, Director of the Cancer

Chemotherapy Center, Japanese Foundation for Cancer Research in Tokyo, has continued to make progress and to contribute to the Division of Cancer Treatment program. Activities encompass drug collection for screening, drug screening and evaluation, monitoring the new drugs undergoing preclinical and clinical trial, clinical investigations, information exchange and overall liaison activities.

The data collected in the screening program continue to be entered routinely into the DCT computer program and active drugs are made available to the DCT. They are collecting on the order of 35 - 40 materials per month including synthetics and natural products.

In the period June 20, 1979 through June 19, 1980, 468 samples (JCI numbers 2044 - 2511) were collected from 34 institutions. There were 67 natural products of which 11 were active. 390 synthetics were tested, of which 73 were active. 27 of these actives were platinum derivatives, while 46 of them were of a variety of structures. The activity observed was in the P388 and/or L1210 systems. 1927 tests were conducted in the P388 and/or L1210 systems and the data were submitted for processing to Value Engineering Company.

The following types of compounds demonstrated antitumor activity (T/C $\geq$ 150 percent in the experimental systems.

A. Synthetics

- | | |
|---------------------------|-----------------------------------|
| 1) cyanines | 2) acylamino 1,3,4,-thiadiazole |
| 3) amidine hydrazones | 4) imidazolyl carboxamide analogs |
| 5) 5-fluorouracil analogs | 6) cytosine arabinoside analogs |
| 7) nitrosoureas | |

B. Natural products

- 1) fatty acid esters of crude nagilactone mixture
- 2) anthracycline antibiotic

Of the samples listed above, 5-carbamoyl-1H-imidazol-4-yl-1-adamantane-carboxylate and 5-carbamoyl-1H-imidazol-4-yl piperonylate are currently in further investigation in a number of animal tumor systems.

In the period June 20 - September 19, 1980, 176 samples (JCI 2512-2687) were received from 12 institutions. There were 12 experimental runs in the P388 system (control PS 0240-0251) and one experimental run in the L1210 system (control LE 0112). Among the samples tested during this period, an anthracycline antibiotic showed high activity against P388 leukemia. Three additional compounds, JCI 2534, 2591 and 2604 were active in the P388 system.

In the period September 20, 1980 to March 19, 1981, 15 additional materials were active in the P388 system.

Information exchange continues to be highly useful in keeping DCT updated on preclinical and clinical activities in Japan. This has included provision of translated titles of abstracts from Japanese (about 50 per month) pertaining to anticancer agents and cancer therapy, from pharmaceutical and medical journals in Japan. In addition, in response to requests from DCT staff, specific abstracts have been made available. Selected publications of interest are also

submitted to DCT.

Information submitted to DCT includes: (1) Reports on scientific meetings; (2) Summarized clinical data and papers; (3) Titles of Japanese papers and provision of abstracts.

During the period June 20, 1979 - June 19, 1980, information submitted included:

- (A) Titles of abstracts - 850.
- (B) Abstracts supplied following request from NCI - 140.
- (C) Clinical data or papers - 6.

These included summarized clinical data or papers selected by Dr. Ogawa on the subjects:

- 1) Current Status of Nitrosoureas being developed in Japan
 - 2) Clinical Study of Aclacinomycin A
 - 3) UFT Combination
 - 4) Phase I Study of a New Antitumor Drug, 1-Hexylcarbamoyl-5-Fluorouracil (HCFU) by Oral Administration
 - 5) A Phase I-II Study of 4-Hydroperoxyisophosphamide
 - 6) Summary of Phase II Clinical Study of Aclacinomycin A
- (D) Report of meeting - 1. This was a pilot study group meeting on fibroblast interferon.

45 scientific meetings were attended in relation to the above activities.

In the period June 20 - September 19, 1980, 247 items of information were obtained including:

- A. Proceedings of the 28th Annual Meeting of the Japanese Society of Chemotherapy (including all titles on cancer chemotherapy) - 45
- B. Summarized clinical papers - 1
- C. Titles of abstracts - 181
- D. Abstracts sent following the requests from NCI - 20

14 scientific meetings were attended in relation to the above activities.

Included were Phase II group meetings involving ACNU, pepleomycin, behenoyl Ara C (BHAC), vindesine and GANU. There was also participation in a Phase II-III study group meeting of Cis-platinum and a Phase III meeting of bestatin.

In the period September 20, 1980 to March 19, 1981, 359 titles of abstracts were submitted and 33 abstracts provided in response to requests. Included also were:

- 1. Proceedings of the 39th annual meeting of the Japanese Cancer Association.
- 2. Clinical papers entitled "Current status of nitrosoureas under development in Japan" and "An overview of chemotherapy for advanced gastric cancer" written by Dr. Ogawa (sent to Dr. Macdonald).

3. "High dose intermittent 6-thioguanine. Phase I and preliminary Phase II observations."
4. Abstracts of presentations on interferon in the annual meeting of the Japanese Cancer Association.

Continued progress is being made in the clinical program, which has been conducted essentially with Japanese funds, as their input to the cost-sharing in this contract. Dr. Makoto Ogawa is a leading Japanese clinical investigator and his vigorous activity has filled an important gap which has transformed the clinical program into a focal center for our clinical collaboration and liaison in Japan.

Sixty-one beds in the Cancer Institute Hospital are available for the clinical research of the Division of Clinical Chemotherapy of the Cancer Chemotherapy Center, and 9 doctors are engaged with Dr. Ogawa in the clinical trials both with in-patients and out-patients.

During the last year, Phase I studies were conducted with N⁴-behenoyl-cytosine arabinoside, the nitrosoureas GANU and MCNU, pepleomycin, neothramycin and fibroblast interferon. Phase II - III studies were conducted with DTIC, cis-platinum, VP-16 and m-AMSA.

Dr. Makoto Ogawa as clinical director of the contract has been in close contact with Dr. Macdonald. He also attended the NCOG group meeting held in San Francisco in October 1979, representing the Japanese side of the NCOG-Japanese Gastric study.

Periodic exchange of information of clinical data of newly developed antitumor agents in Phase I and II trials has been continued.

The following protocol studies are underway with investigational drugs under NCI sponsorship.

A. 6-Thioguanine

For the combination chemotherapy of acute non-lymphocytic leukemia.

B. DTIC

For the treatment of malignant melanoma and sarcoma.

C. BCNU

For the treatment of gastric cancer in collaboration with the U.S. - Japan Joint Study for Advanced Gastric Cancer.

D. VP-16-213

For the treatment of small cell carcinoma and lymphoma.

E. m-AMSA

For the treatment of acute non-lymphocytic leukemia refractory to previous treatments.

The protocols are outlined in the Progress Report of June 20, 1979 - June 19, 1980. During the same period there were a total of 4434 out-patients (370 per month) and a total of 229 in-patient admissions.

This contract continues to be in close coordination with the contract of Dr. H. Umezawa (Dr. J. Douros, project officer) particularly in relation to natural products. The appointment of Dr. J. Douros as Co-Project Officer on this contract has facilitated the coordination of the two contract activities in relation to the total therapy effort in Japan and the input to DCT.

The two contract activities fortify each other, and because of the prestigious status of the investigators and their formal association with the NCI, an entree continues to be provided to DCT into essentially all of the cancer therapy and associated activities in Japan.

USSR

The collaborative monograph "Experimental Evaluation of Antitumor Drugs in the USA and USSR and Clinical Correlations" has now been published as Monograph 55 of the National Cancer Institute. The Russian version has also been published. The English and Russian editions have been interchanged between the two countries.

The third USA-USSR meeting on Breast Cancer Treatment and Anticancer Drug Evaluation was held May 21-23, 1980 at the National Cancer Institute in Bethesda.

At the working meeting there were reviews of (a) the study employing tamoxifen in the treatment of breast cancer; (b) Proceedings of the Sixth USA-USSR Meeting on the treatment of lung cancer; (c) the testing of exchange clinical anticancer drugs; (d) update of the USSR studies with prosperdine. There was also discussion of drugs for future exchange for clinical trial and it was agreed that specific drugs would be interchanged.

For breast cancer the USA and USSR participants discussed the problems of combined treatment of stage III - IV breast cancer. The final version of the Joint Clinical Protocol on Post Operative Prevention of Relapses and Metastases of Stage II B Breast Cancer after radical surgery was clarified and coordinated. At the end of 1979 Tamoxifen was received from the USA for trial III of the Joint Program and at this time 32 patients were involved in this trial. There was agreement to discuss the role and place of irradiation in combined modality treatment of breast cancer at the next meeting together with the principles of the management of infiltrative breast cancer and at that time to delineate the protocols on the treatment of disseminated breast cancer. The results of American and Soviet scientists on a joint clinical program in preventive chemotherapy of Stage II breast cancer is of a preliminary nature and it was agreed that this study be continued. Overall the American and Soviet scientists exchanged detailed information on new directions in breast cancer treatment and it was agreed that the next working group meeting be held in 1981 in the USSR.

The experimental results with exchanged preclinical drugs were presented. This included data on 107 drugs received in the USSR exchange program. Of these, prosperdine clearly met all of the criteria for activity and novelty with respect to further preclinical development. 4 additional drugs, palphicerin, fotrin, dioxadet and olivomycin A, although definitively active in the USA experimental

tumor systems are not being pursued further at this time.

New American and Soviet Preclinical drugs for potential interchange were discussed and plans made for exchange of various ones of these agents.

The present status of the NCI Drug Development Program, including the nude mouse studies was presented. Additional items included: Exchanges on biochemical pharmacology and plans for the forthcoming USA-USSR workshop; Soviet studies with drug evaluation and canine species; the American experience and plans for biological response modifiers; the Soviet experience with biological response modifiers; the prospectus for an immunotherapy meeting.

A symposium on "Biochemical Pharmacology as related to the Clinic" organized under the USA-USSR Agreement was held October 26-28, 1980 at the Oncological Scientific Center of the Academy of Medical Sciences in Moscow. The papers presented by both sides were clearly of high calibre and provided the Americans an overview and updating of the program in the Soviet Union in clinical biochemical pharmacology and related areas of cancer investigation. The Soviet investigators, in broadening the scope of the symposium, brought in leading investigators from a number of institutions with presentations of novel and interesting approaches and projects for potential collaboration. There was considerable emphasis on the Soviet side throughout the symposium on the desirability of continuation of collaboration and on interchange of scientists. The range of topics is illustrated by the program of the Symposium as follows:

AMERICAN-SOVIET SYMPOSIUM ON "BIOCHEMICAL PHARMACOLOGY
RELATED TO CLINIC"

CRS AMS USSR, 26-28 October 1980

Opening Ceremony

Welcome

"Focus and Goal of the Meeting"

E. Mihich (USA)

Chairpersons: A.M. Garin (USSR), Mihich (USA)

"Clinical Concepts Derived from Animal
Experimentation"

A. Goldin (USA)

"The Prognosis of Spectrum of Action of Antitumor
Drugs on the Basis of Experimental Data"

T.G. Glazkova (USSR)

"The Influence of the Scheme of Drug Implementation
on the Treatment and Toxicological Effects"

Z.P. Sofjina (USSR)

N.A. Lesnaya "

N.A. Babushkina "

N.P. Konovalova "

E.G. Kiseleva "

"The Influence of Regimens of Drug Administration
on Metastatic Process"

P.F. Djatchkovskaya "

L.M. Volkova "

E. Modest (USA)

"The Modification of Tumor Sensitivity Profile
by Drug Regimens:

"Biochemical Aspects of Metabolism and Pharmacokinetics of Ftorafur"

A.P. Gilev (USSR)

Discussion

Z.P. Sofina (USSR)

II. Session Chairpersons: N.I. Perevodchikova (USSR), E. Modest (USA)	
"Mechanism of Antitumor Action of Nitrosoureas"	L.B. Gorbacheva (USSR)
	V.N. Verovsky "
	I.S. Sokolova "
	G.V. Kukushkina "
"New Data on Mechanism of Anthracycline Action"	Y.V. Dudnik (USSR)
	G.F. Gauze "
"Cellular Pharmacology of Anthracyclines in Humans"	P. Kanter (USA)
"Pharmacological Studies of Anthracyclines"	L.E. Goldberg (USSR)
"Role of Latent DNA Damage in Mechanism of Action of Alkylating Compounds"	V.A. Struchkov (USSR)
"Activity of Non-Specific Liver Oxidases and Biological Effects of Antitumor Drugs"	T.A. Bogush (USSR)
	A.B. Syrkin "
	A.F. Buchny "
	L.A. Durnov "
	F.V. Donenko "
	A.I. Saltanov "
	G.Y. Seitlin "
"Phagocytosable Depot Forms of Anticancer Drugs and Metabolites"	M.N. Preobrazenskaya (USSR)
	V.M. Bukhman "
	N.A. Brusentsov "
	N.A. Lesnaya "
	Z.P. Sofina (USSR)
	P. Kanter (USA)
Discussion	

III Session A.K. Belousova (USSR), Y.M. Rustum (USA)

"Determinants of Antimetabolite Action in Humans"	Y.M. Rustum (USA)
"Determinants of Acute Leukemia Sensitivity to Cytostatics"	G.K. Gerasimova (USSR)
"Thymidine Metabolism in Hepatomas and Lymphoid Tissues in Host"	G.I. Vornovitskaya (USSR)
Discussion	
"Modification of Ara-C, 5-Fu and MTX Therapy"	E. Cadman (USA)
"Modification of Antitumor and Toxic Action of Antimetabolites by New Thymidine Derivatives"	G.A. Belitsky (USSR)
"Modification of Biological Effect of Ara-C by Deoxycytidine"	V.M. Bukhman (USSR)
	M.F. Barkhotkina "
	N.I. Belyanchikova "
	B.D. Brondz "
	A.A. Pimenov (USSR)
	E. Cadman (USA)
Discussion	

IV Session. Chairpersons: A.B. Syrkin (USSR),
K. Osborne (USA)

"Receptors and Hormone Therapy"	K. Osborne (USA)
"Receptors of Steroid Hormones and Prognosis of Effective Endocrine Therapy"	L.S. Barsalyk (USSR) V.V. Vishnyakova " N.I. Muravjeva "
"Molecular Mechanism of Regulation of Breast Tumor Growth by Androgens"	T.I. Merkulova " T.M. Morozova " P.I. Salganic "
"Molecular Mechanisms of Hormonal Cytostatic Action"	A.K. Belousova " O.V. Boguslavskaya " V.V. Bergolts "
"The Role of Endocrine Mechanisms in Antitumor Activity of Hormonal Cytostatics"	M.D. Lagova (USSR)
Discussion	A.K. Belousova (USSR)
V. Session. Chairpersons: M.N. Preobrazenskaya (USSR) A. Goldin (USA)	
"Inhibitors of Serinhydroxymethyltransferase as Antimetabolite of Folic Acid"	Y.V. Bukin (USSR)
"Mechanisms of Enhancement of Antitumor Activity of Methotrexate by Cobalamine Derivatives"	N.V. Myasicheva (USSR)
"Biochemical Determinants (Targets) of Inhibitors of B ₁₂ -dependent Enzymes"	A.M. Yurkevich (USSR)
"Antioxidants in Cancer Chemotherapy"	E.B. Burlakova (USSR)
Discussion	Y.V. Bukin (USSR)
"Progress under the USA-USSR Agreement"	A.M. Garin (USSR)
"Summary of Symposium"	E. Mihich (USA)
"Final Remarks"	A. Goldin (USA)

ITALY

There were two primary events during the year on the USA-Italy Agreement in the period October 29-November 1, 1980.

- a. A Business Meeting held in Stresa
- b. A Workshop on Clinical Biochemical Pharmacology held primarily in Stresa with a final session, open to the public, held at the Mario Negri Institute in Milan.

At the Business Meeting there was a review of research activities going on in Italy and the USA which could provide a basis for collaborative research between the two countries.

The characteristics of the cooperative relationships were defined as follows:

Categories

1. Fully cooperative activity (e.g., joint clinical trial).
2. Associated activity: common problem but developed separately in the two countries.
3. Exchange of information (ranging from exchange of protocols and related results, to exchange of new leads and ideas of both preclinical and clinical nature).
4. Exchange of personnel (e.g., basic training, special techniques training, data base and statistical expertise, etc.)
5. Exchange of materials (e.g., tumor models, reference compounds, agents for study, etc.)
6. Workshops

Programs

Six programs were selected for collaboration. They are tabulated below with their assigned category and the names of the coordinators plus those of the collaborators who are to be involved in them.

A. Studies of breast cancer (Bonadonna and Fisher)

Stages I-II: categories 2 and 3

Stage III: category 1

B. Pediatric Studies (Massimo and Simone; with the involvement of Garattini, Henney and Pizzo)

- a) Pain (Gaslini): Category 3, later 2 (to be developed)
Category 4 (data collection expertise of Memorial, NYC)

- b) Antiemetics: Exchange of protocols and non-controlled drugs
Categories 2, 3 and 5
- c) Information booklet on cancer, by NCI: have it translated in
Italy and printed: Categories 3 and 5
- d) Phase II studies of deoxycoformycin (Gaslini and St. Jude)
Categories 2, 3 and 5
- e) AML Protocols: Category 3
- f) Brain tumors: ground work to assess resources, needs and
feasibilities in the two countries, leading to a Workshop and
later to joint programs. Category 3 (plus task force)
- C. Clinical Biochemical Pharmacology Studies (Mihich and Veronesi;
with the involvement of Chabner, Rustum, Kanter, Silvestrini,
San Filippo, Garattini, Mantovani and D'Incalci)
 - a) Parameters of drug action - Categories 2, 3, 4 and 6
 - b) Soft agar colony test: Categories 2, 3, 4 and 5
 - c) Cell desegregation and suspension from solid tumors; methodologies:
Categories 2, 3, 4 and 5
 - d) Metabolic modulation: Categories 2, 3, 4 and 5
- D. Studies of Metastasis Models (Mihich and Garattini; with the
involvement of Bernacki, Bertram, Spreafico) - Categories 3, 4 and 5
- E. Studies of Anthracyclines: Two new analogs selected initially; others
optional (Chabner and Giuliani; with the involvement of Myers, Kohn,
Mihich, Kanter, Rustum, Gessner, Casazza, Formelli, Mantovani):
Categories 2, 3, 4 and 5
- F. Studies of BRM (Macdonald and Bonmasser; with the involvement of Mihich,
S. Carter, Oettgen, Cudkowicz, Goldin, Cannelos, Mantovani, Spreafico,
Nicolin, Gavosto, Bonadonna, Ghione)
 - a) Thymic factors: Categories 2, 3 and 5
 - b) Interferons: Categories 2, 3 and 5
 - c) Monoclonal antibodies: Categories 2, 3 and 5
 - d) Immunomodulation: Categories 2, 3, 4 and 5

In addition, it was agreed that Drs. Pistenma, Macdonald and Cappellini would prepare the ground for a program on irradiation (X-ray, photoradiation, regional hyperthermia) to be proposed for the second year.

At the Business Meeting there was discussion of budgetary matters, agreement on intervisits of scientists and the scheduling of the next meeting and a workshop in November 1981.

The Workshop on Clinical Biochemical Pharmacology was organized in order to bring into focus the opportunities for collaborative clinical biochemical pharmacology studies that may develop under the aegis of the USA-Italy Agreement. This program was highly successful and the range of the presentations and discussion is indicated by the program of the workshop as follows:

USA - ITALY AGREEMENT

WORKSHOP ON CLINICAL BIOCHEMICAL PHARMACOLOGY

Stresa - "Grand Hotel des Iles Borromees" - October 30-31, 1980
Milan - "Mario Negri Institute" - November 1, 1980

Program Committee: E. Mihich and B. Chabner (USA)
S. Garattini and M. Ghione (Italy)

October 30 (Stresa)

Business Meeting - 1st part

Opening of the Workshop - Welcome addresses Garattini, Veronesi

- Focus and goals Mihich

Opening Remarks: Clinical concepts derived from Goldin
animal experimentation

Receptors and hormone therapy Osborne

Estrogen receptors and cell kinetics in human Silvestrini
breast cancer

Studies with new anthracyclines Casazza

Cellular pharmacology of anthracyclines - humans Kanter

Possible biochemical determinants of anthracycline Rausa
cardiotoxicity

Mechanisms of cardiotoxicity Myers

October 31 (Stresa)

Business Meeting - 2nd part

Determinants of Ara C and FU action in humans Rustum

Biochemical evaluation of drug action in human tumors Sanfilippo

The modification of MTX therapy Bertino

The modification of Ara C and FU therapy Cadman

Determinants of platinum complexes

Kohn

Determinants of nitrosoureas

Schein

The modification of tumor sensitivity profile
by drug regimens

Modest

General discussion

November 1 - Mario Negri Institute, Milano

V.T. DeVita (Dr. J. Macdonald) - New antitumor drugs

S. Garattini - Pharmacokinetics in cancer therapeutics

E. Mihich - BRM: New vistas in pharmacology and therapeutics

LATIN AMERICA

The USA-PAHO-Latin American Collaborative Cancer Treatment Research Program continues to make steady progress. On March 17, 1980 a Progress Review Meeting was held at PAHO with the attendance of all of the U.S. principal investigators and NCI staff. The agenda was as follows:

1. General review of progress and future activities

- Cooperating groups
- Exchange of junior investigators and nurses
- Publications, abstracts
- New centers, etc.

2. Protocol Activity

- Preparation format
- Notification of withdrawal, closure
- Joint protocols
- Final reports
- Multi-center participation

3. Advisory Scientific Review Committee

4. Discussion of the Bogota June Meeting

The meeting was highlighted by an extensive discussion on the role, functions modus operandi and composition of the proposed scientific review group.

The Second Annual Meeting of the Collaborative Cancer Treatment Research Program (CCTRP) and the associated Business Meeting of the USA-PAHO Latin American Program, June 18-20, 1980 was held in Bogota, Colombia.

The objective of the program review was to present and assess the status of the ongoing protocols and to plan further investigations of the USA-PAHO-Latin American Program. Four major areas were covered at the second annual CCTRP meeting and the protocols reviewed in the subject categories within each major area are as follows:

I. Therapeutic concepts in Hematologic Malignancies:

- a) New agents in lymphomas and leukemias: 4 protocols
- b) Staging and treatment planning in Hodgkin's disease: 1 protocol
- c) Chemotherapy of Non-Hodgkin's disease: 1 protocol

II. Therapeutic concepts in Childhood Malignancies, Osteosarcomas and Testicular Cancer:

- a) Childhood malignancies: 4 protocols
- b) Medulloblastoma: 1 protocol
- c) Testicular cancer: 1 protocol

III. Multimodal Concepts in Solid Tumors:

- a) Solid tumors: 2 protocols
- b) Head and neck cancer: 4 protocols
- c) Breast cancer stage III: 1 protocol

IV. Systemic Therapy of Solid Tumors:

- a) Advanced breast cancer: 3 protocols
- b) Advanced gastric cancer and adenocarcinoma: 4 protocols
- c) Sarcomas and other tumors: 2 protocols

All of the ongoing protocols were subjected to detailed discussion as to status of patient accrual, results, any attendant problems and future planning. In addition to the Rapporteur's comments and discussion for each subject, a constructive feature of the program included panel discussions in three pertinent areas:

- a) Adjuvant therapy of osteosarcoma
- b) Multimodal approach to cervical cancer
- c) Multimodal approach to head and neck cancer

Dr. Julio Ospina, Director of the Instituto Nacional de Cancerologia, Ministerio de Salud Pública, Bogotá, Colombia, represented the host country for this program and also extended an invitation for participation in a two day symposium on Breast Cancer of which I attended the second day.

An important recommendation stemming from the meeting was the feasibility of multinational joint studies, involving a number of Latin American countries in collaboration with the USA on specific protocols, in order to increase patient accrual in broad scale studies.

It was stressed that individual types of cancer may differ in their growth behaviour and response to treatment in different countries and the development of programs that extend beyond treatment, to provide information and answers concerning etiology and epidemiology, was considered highly appropriate.

There have been interesting and significant advances in this cooperative program between Latin American and USA Centers.

The Business Meeting was attended by all of the representatives of the participating countries.

A document was prepared and distribution made by the Operations Office of PAHO for the second annual review, and the business meeting. Discussion based on this document, and other matters discussed at the meeting include the following:

- a) An historical summary of the USA-PAHO Latin American Program. The program is entering the third year of operation and has been successful in developing true collaboration among the participating centers.
- b) Copies of abstracts presented in meetings including ASCO and others and also papers submitted for publication in various journals. In this regard it was agreed that listings of publications, abstracts of manuscripts, and titles of papers presented under the program be submitted within a month in order to complete the listing for the annual report, and on a continuing basis thereafter.
- c) The complete list of protocols. During the last year six new protocols were incorporated into the program. Three of the protocols include new associated centers.
- d) Guidelines for the presentation of protocols.
- e) Distribution of patient accrual. Up to April 30, 1980, the program accrued 1158 patients and in the last year, since May 1979, 618 patients. The participating Institutes are the same 9 centers that were active at the beginning of the program. However, there are 45 Institutes and 92 investigators who are participating. Thus, the participating centers in the individual countries include Institutes that are providing patients and further expansion within that country, an activity which has been encouraged since the initiation of the program.
- f) Listing of physician and nurse exchanges that have taken place, with support of the program. In addition, because of the existence of the program, additional exchanges have taken place, related to the program, but with other funding. The exchanges have been highly useful in furthering program activities, and additional exchanges are planned.
- g) Anticancer drug shipments. The minimum lead time for drug shipments is one month, to cover material for three months. In emergency situations special arrangements can be made through PAHO.

h) Budgetary statements and requests for PAHO for purposes of contract renewal and other budgetary considerations including the need for economies.

i) Consideration of protocols. Two major protocols and two pilot protocols per institution serve as a guideline. Institutes are developing independent protocols and the associated protocol concept is acceptable. The development of multinational joint protocols encompassing groups of institutions involving more than one Latin American country is under consideration. Cervical and gastric cancer may be suitable for this purpose because of relatively high incidence in various ones of the Latin American countries.

j) A program review committee has been constituted, to review the performance of the individual Institutes on all of the ongoing and proposed projects. The review group is advisory to the Clinical Oncology Review Committee of the Division of Cancer Treatment, NCI and to PAHO in relation to performance and budgetary allocations. The review group includes an executive secretary who is also the executive secretary of the clinical oncology review committee, three ex officio members, four U.S.A. coordinators of the USA-PAHO-Latin American Program, three NCI members and two outside reviewers who are also members of the Clinical Oncology Review Committee. Committee assignments will be for one to two years, so that there will be a rotation of membership of all of the current U.S.A. program participants. The program review will be based primarily on four aspects:

1. The type of protocol development
2. The ability to accrue patients into the protocols
3. The quality of performance
4. Other contributions that the institution is making to the program, including attendance at meetings, publications, and so forth

k) It is planned for the next annual program review to be held in conjunction with the ASCO and AACR meetings, including a scientific session possibly on cervical cancer.

As part of the business meeting there were summary presentations and discussion of accomplishments and problems pertaining to each of the projects. Overall it is clear that the past year was one of continued growth, with resolution of any problems in the various defined areas of collaboration. Many of the protocols have been successful and are being continued and some are being replaced by more appropriate protocols with more promise of better patient accrual. The quality of the data has been high, the scientific exchanges are being successful and the studies are being reported at both international and national scientific meetings and are being published. The prospects for further accomplishment in this program are excellent.

One afternoon was devoted to a visit to the facilities of the Instituto Nacional de Cancerologia, hosted by the Director, Dr. Julio Ospina.

FEDERAL REPUBLIC OF GERMANY

The USA-Federal Republic of Germany (FRG) Bilateral Agreement protocol for Drug Development and Testing Program for Cancer Therapy (DDP) is now in the final

stages of implementation. The USA-FRG Bilateral Agreement consists of seven projects and at a joint meeting on October 20, 1980, in Bethesda, coordinators were selected for each area. The projects are as follows:

1. Exchange of information and material

Coordinators: NCI: Goldin, Yoder, Macdonald
DDP: Brock, Hohorst, Schmahl, Schmidt

2. Broadening of spectrum of tumor screening including relevant models of the German and American sides

Coordinators: NCI: Venditti
DDP: Brock, Zeller

3. Human tumor xenografts

Coordinators: NCI: Venditti, Goldin
DDP: Osieka, Fiebig

4. Differentiation protection

a. Loco-regional chemotherapy

Coordinators: NCI: Plowman, Zaharko
DDP: Hohorst, Brock

b. Regional detoxification

Coordinators: NCI: Venditti, Macdonald
DDP: Brock, Klein

5. Water-soluble and other pharmaceutically suitable nitrosoureas - study of chemotherapeutic and long-term toxic effects.

Coordinators: NCI: Narayanan, Kohn
DDP: Eisenbrand, Schmahl, Osieka

6. Phase I and II studies

Coordinators: NCI: Macdonald, Hoth
DDP: Schmidt, Klein

7. Personnel Exchange

Coordinators: NCI: Goldin, Schepartz, O'Connor
DDP: Brock, Hohorst, Schmahl, Schmidt

Considerable momentum has been established for this program and the prospects for successful implementation are excellent.

The DCT program continues to have broad contacts in West Germany with pharmaceutical houses, chemical industry and universities and this has been providing important input of candidate antitumor agents.

UNITED KINGDOM

Cooperative preclinical and clinical research programs between the USA and the United Kingdom continue to be largely self-generated, with additional stimulation being provided by the National Cancer Institute and Division of Cancer Treatment. These continue to encompass a) drug development and screening; b) preclinical toxicology; c) clinical Phase I-II testing; d) drug rescue strategies and scheduling; e) collaborative pharmacological and clinical testing of new drugs.

The close association with the Institute of Cancer Research, particularly with the program of Dr. K. Harrap continues to be fruitful. Specific arrangements are currently in progress for the conduct of preclinical toxicologic evaluation and Phase I studies with selected analogs of known antitumor agents being studied in the U.S. The activities on contract No. 1C-CM-43736 with the Institute of Cancer Research (K. Harrap) with J. Venditti as Project Officer continues to contribute significant data. Cooperative program on chromatin as a target for nuclear-reacting drugs and the significance of DNA repair processes in cancer chemotherapy constitute areas of important cooperative program in both the U.S.A. and U.K., and visits of Doctors Tew and Smulson to the laboratory of Dr. K. Harrap have contributed in this direction. The interchange between the U.S.A. and U.K. is highly active on a continuing basis and is kept so by frequent inter-visits and attendance at both international and national meetings.

HUNGARY

Although the U.S.A.-Hungarian Cultural and Scientific Agreement program document was signed in October 1979 to cover the years 1980-81, specific implementation of the cancer segment of the program has been slow in coming. Suggested projects for the USA-Hungary Collaboration and Cancer Treatment research have included:

- 1) Exchange of candidate antitumor agents including synthetic and natural products:
 - a) new structural classes
 - b) analogs of special interest
 - c) drugs of proven interest
- 2) Collaborative testing of selected exchanged agents in tumor test systems in both countries, including standard systems common to both countries and in unique systems including human tumor xenografts growing in athymic mice. Testing of drugs in a spectrum of human tumors such as colon, lung, breast or other types of solid tumors, with results related, where possible, to patient responsiveness.
- 3) Fundamental preclinical and clinical biochemical - pharmacological and biological investigation of individual drugs, drug combinations including metabolite - antimetabolite interactions and combined modalities with a view to optimization of clinical therapy.
- 4) Investigation of selective delivery systems such as liposome encapsulation of antitumor drugs.
- 5) Fundamental studies of the process of metastasis. Investigation of means for prevention and therapy of the various stages of the

metastatic progression.

- 6) Preclinical toxicologic characterization of drugs employing protocols which will serve as a basis for clinical application.
- 7) Cooperative Phase I & II clinical trials with exchange drugs.
- 8) Investigation of selected areas in Biological Response Modification of therapeutic pertinence including projects such as (a) alteration of tumor cell antigenicity and therapeutic application in conjunction with chemotherapy; (b) biological investigation of antigen-antibody immuno complexes in tumorous patients; fundamental studies and therapeutic applications of plasmapheresis in patients with malignancy (c) special studies such as characterization of "alloantisera" specific for T cell sub-populations; production and investigation of the action of monoclonal antibodies against transplantation antigens of lymphocyte sub-populations (d) influence of biological response modification on the metastatic process.

Exchanges of personnel are requisite for the accomplishment of agreed upon projects.

There has been the suggestion that there be an annual scientific review workshop and adjunct business meeting.

PEOPLES REPUBLIC OF CHINA

The USA-PRC cooperation in Cancer Research is still awaiting specific implementation. In the cancer treatment area two primary topics encompass:

1. Comprehensive therapy of primary forms of malignant tumors employing individual and combined modalities of treatment including chemotherapy, surgery, radiation and immunotherapy. 2. Screening, pharmacologic and toxicologic evaluation and clinical testing of selected anticancer drugs of potential use in the treatment of localized and disseminated forms of human cancer. Specific areas for implementation include:

- a) joint research on the treatment of cancers of the esophagus, lung and hepatic system using individual and combinations of treatment
- b) exchange of experience and information on the treatment of nasopharyngeal, gastric and breast cancers as well as choriocarcinoma with an outlook toward future joint research
- c) exchange of anticancer preparations - conventional chemotherapeutic agents, herbal derivatives and products of natural and synthetic origin for experimental investigation
- d) preclinical and clinical testing of anticancer drugs according to mutually acceptable protocols.

The second meeting of the NCI Steering Committee for collaborative cancer research with the People's Republic of China was held December 18, 1980 for discussion of specific items pertaining to the cancer research workplans which

evolved during the 17-20 November 1980 meeting of the USA-PPC Joint Committee for Medicine and Public Health; the exchange of scientists and overall budgetary plans. In essence, all of the basic planning has been done so that it would be possible to move quickly as soon as the opportunity presents itself to go ahead.

Dr. Bin Xu, Chairman and Professor of the Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences in Shanghai, China, visited the DCT for a month, with his program coordinated through the Office of the Assistant Director for International Treatment Research. In addition to discussions with various investigators in the Division of Cancer Treatment, he presented a seminar on "Recent Progress in anticancer drug research in the People's Republic of China". This visit provided a good opportunity for information exchange and an attempt is being made to arrange for exchange of drugs for preclinical testing on a scientist to scientist basis with Dr. Bin Xu and his colleagues of the Institute of Materia Medica CAS, Shanghai.

POLAND

An exchange visit of Dr. Czeslaw Radzikowski to the U.S.A. is in the process of being arranged. It is felt that this visit will provide additional means for implementation of bilateral program.

BULGARIA

There is continuation of contact with Bulgaria particularly through Dr. J. Stoychkov who spent a Fellowship year at our Institute and Drs. G. Mitrov, and D. Toderov of the Bulgarian Cancer Institute.

CZECHOSLOVAKIA

There is continued maintenance of contacts for information and drug exchange especially with Prague and Bratislava.

EGYPT

The bilateral program between the USA and the Republic of Egypt under the leadership of Drs. J. Macdonald and J. Ziegler continues to have steady accrual of patients. Dr. J. Macdonald visited the Cairo Cancer Institute with an excellent opportunity to view the whole program of Dr. Nazli Gad-El-Mawla, Medical Oncologist at the Cairo Cancer Institute. He also met there with the visiting U.S. medical oncologist, Dr. C. Stettin. It is considered that the Phase II data that is being obtained from our cooperation with the Egyptians has been quite good and certainly there is no other area of the world where we could have obtained the information on squamous cell bladder cancer that has become available through this program. The Phase II data that is being obtained in the collaboration are valid findings of carefully done clinical studies and it is planned for this cooperative effort to be continued.

HONORS AND AWARDS DURING THE YEAR

None.

The following talks on aspects of the Division of Cancer Treatment Program were given during the year by A. Goldin:

1. George Washington University Pharmacology Department

"Clinical Concepts Derived from Animal Chemotherapy Studies."

2. Sidney Farber Cancer Institute

"The Current Status of Screening as Viewed at the Division of Cancer Treatment, National Cancer Institute."

3. EORTC Metastasis Conference at the Royal Institution, London

"Potential for Selective Inhibition of Metastases."

4. U.S.-Japan Cooperative Cancer Research Program Treatment Review Meeting

"The Current Status of NCI Screening."

5. Paris - EORTC Annual Meeting

"Biological Response Modifiers and Adjuvant Chemotherapy."

6. USA-USSR Cooperative Agreement for cancer research in Moscow, USSR

"Clinical concepts derived from animal experimentation."

7. USA-Italy Agreement in Stresa and Milan, Italy

"Clinical concepts derived from animal experimentation."

8. Joint Meeting of the British Association for Cancer Research (BACR) and the Section of Oncology of the Royal Society of Medicine, in London

"Selection, evaluation and development of antitumor drugs and the relevance to the clinic."

9. INSERM-NCI International Symposium on Nitrosoureas in Cancer Treatment in Montpellier, France

"Historical overview of nitrosourea development."

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ANNUAL REPORT - SCIENTIFIC INFORMATION BRANCH
1980 - 1981

The Scientific Information Branch was established in 1980 to provide over-all management and direction to the publications of the DCT and its information gathering and reporting services. The SIB is located in the Office of the Director, DCT, NCI and contains two major components: the Publications Section and the Literature Research Section. Its activities can be divided into three major areas: the publication of scientific and technical information in Cancer Treatment Reports, Cancer Treatment Reports Symposia, and the DCT Bulletin; the preparation of information surveys, reports and reviews for the scientific staff of the DCT and its committees and panels; and the operation of the DCT library. In addition, the Chief, SIB, participates with senior investigators of the Medicine Branch in the evaluation and analysis of various ongoing clinical trials.

PUBLICATIONS

Cancer Treatment Reports (CTR), a primary source scientific journal dealing with preclinical and clinical cancer treatment is in its twenty-second year of continuous publication. From 1959 to 1968 CTR, then known as Cancer Chemotherapy Reports, was issued 6-10 times a year depending on the acceptance of manuscripts submitted. Several types of manuscripts were published, including program information, study protocols, experimental studies, and clinical reports. Most but not all manuscripts were reviewed by outside referees as well as by the editorial board. In 1968 the journal expanded to three parts: Part I (original research, both experimental and clinical), Part 2 (comprehensive, lengthy chemotherapy studies involving a great deal of tabular material), and Part 3 (program information including study, protocols, clinical brochures, toxicology reports, and review articles).

In January 1976, the journal was renamed Cancer Treatment Reports (CTR), dropped the three part separately numbered system, and began monthly publication. The journal now considers unsolicited and previously unpublished manuscripts of original work under seven major categories:

1. Full length manuscripts containing the results of clinical or preclinical research.
2. Brief Communications
3. Letters to the Editor
4. Clinical Trials Letters
5. Guest editorials and commentaries
6. Current Controversies in Cancer Management
7. Meeting reports

All material submitted for consideration in CTR is subject to review when appropriate by two outside reviewers and a member of the editorial board. In addition, the journal publishes meeting announcements and program information for oncology programs throughout the world.

Submissions in 1980 and 1981

During 1980, the journal received 576 research manuscripts and 98 symposia manuscripts. During the first 5 months of 1981, 298 manuscripts have been submitted.

Comparative data on the CTR series of publications are shown in table 1 on the following page.

Publication of monthly issues of CTR was severely compromised by the loss of its managing editor and the fact that the Publications Section of CTR was relocated into new office space twice during the year. These difficulties created a serious backlog in the publication of CTR. Reorganization of the Publication Section into the new Scientific Information Branch has led to the resolution of many of the administrative and support problems that contributed to the backlog. During its first six months, the SIB has initiated the following actions to improve operations:

1. Reorganization of staff to provide additional support to the managing editor of CTR and to the operations of the editorial office.
2. Conversion of the office operations to word processor automation containing a record management system that will facilitate efficient processing, indexing, and tracking of submitted manuscripts.
3. Revision of the review process to expedite rapid reviews and decisions on submitted manuscripts.
4. Separation of the publication of symposia from the publication of refereed papers and application for separate contracts for publication and support of symposia. This will permit the SIB to resume regular and timely publication of original manuscripts in CTR during 1981.
5. Introduction of a new section of the journal that contain summary data from negative clinical trials in 300 word communications.

Sections of the Journal

Brief Reports - In 1973, a section entitled Brief Reports and Preliminary communications was added to the journal. This section contains short clinical manuscripts and abbreviated reports of preliminary research. Brief Reports can be processed by the Publications Section more quickly than full length manuscripts. They are cost efficient in terms of editorial effort and journal space and increase the number of research studies that can be disseminated to the readership. Submissions to this section continue to be excellent.

TABLE 1

Year	Manuscripts Received	Manuscripts Accepted	Issues Published	Pages Published	Reviewers	Total Costs (\$)*	Cost Per Page (\$)
1973	244	207	7	1100	167	59,897	54
1974	286	180	11	1823	213	72,325	40
1975	365	261	9	1888	295	95,498	51
1976	478	302	12	2021	402*	89,732	45
1977	422	313	9	1771	432	109,276	61
1978	578	364	12	2168	557	129,232	59
1979	740	667	10	2175	695	138,678	64
1980	576	269	4	827	595	70,187	85
1981	197**	82**	4†	773**	---	Not Available	--

* Includes the cost of paper, printing, typesetting, and distribution only

** From January 1 to May 31, 1981

† 10 issues are scheduled for publication in 1981, 3 back issues for 1980, 6 issues for 1981, 1 issue for 1982

Editorial Commentaries - In 1974, a section containing invited editorials and commentaries was initiated. Authoritative scientists are invited by the editorial board to comment on subjects of current interest in cancer treatment. In the past 7 years, many such manuscripts have been received and published. In 1981, the SIB invited prominent investigators to provide editorial commentaries on the management of patients with non-small cell bronchogenic carcinoma, histologic progression in non-Hodgkin's lymphoma, and statistics in medical research. Pertinent manuscripts will be grouped in the issue by disease and/or therapy for discussion by the guest editor.

Letters to the Editors - During the past year, CTR has observed a significant increase in the number of Letters submitted and accepted for publication. Letters usually refer to previously published articles or represent unique case reports.

Clinical Trials Letters - In January 1981, this new section was introduced. Clinical Trials Letters are brief summaries of clinical trials that have produced negative results. Authors are limited to 300 words of text but are encouraged to present patient characteristics, response data, toxicity, and survival in tabular form. This new format is concise, and can be processed and published quickly and efficiently by the editorial staff. It has permitted publication of results from an increased number of clinical trials that contain essential, albeit negative, research data. The response to this new format and to the Editorial Board's requests for revision and condensation has been excellent.

Current Controversies in Cancer Management - This new section will contain 3 to 4 invited papers by prominent investigators that address controversial aspects of cancer treatment. The following subjects are being prepared for publication in this section:

1. The management of patients with stage I non-seminomatous testicular cancer
2. Randomization in controlled clinical trials
3. Maintenance therapy in acute leukemia
4. The management of patients with nodular non-Hodgkin's lymphoma

Proceedings of Symposia

During the past several years, CTR has been approached frequently to publish the proceedings of scientific meetings sponsored by the Division and, in 1980, published:

1. Proceedings of Symposium on Designs for Clinical Cancer Research
2. Proceedings of Symposium on Antiestrogen Therapy for Hormone-Dependent Tumors

In 1981, the following will be published:

1. International Symposium on Methotrexate
2. Symposium on Nutrition and Cancer
3. The 8th New Drug Seminar on L-Asparaginase and Daunorubicin
4. Third Conference on Brain Tumor Therapy
5. Advanced Seminar on Clinical Biochemical Pharmacology

In 1982, the following symposia are scheduled for publication:

1. Proceedings of the Japanese-American Agreement
2. Symposium on Contemporary Issues in Hodgkin's Disease: Biology, Staging, and Treatment

Editorial Board

The Editorial Board includes the editor-in-chief and twelve associate editors. Each year three editors rotate off the board and three new members are added. Provisions have been made to allow editors to have an additional year on the board at the discretion of the editor-in-chief and the Director, DCT. An Advisory Editorial Board of 15 members has also been established to supplement the areas of expertise represented by the associate editors. The official policies of the journal are contained in the official charter which was established in 1975.

Coverage of CTR in Current Contents and Related Publications

Since 1967, CTR has been listed in Current Contents, Life Sciences. In 1973, CTR was included in a new publication of current titles in collaboration with Science, Engineering, Medical and Business Data Ltd., Oxford, England. The Japan Medical Service, which publishes a supplement to its Index of Japanese Medical Periodicals listing foreign publications also includes CTR. This additional coverage of material presented in the journal has increased the demand for subscriptions, particularly in other countries.

In 1964, CTR began sending copies of each issue to the Chemical Abstracts Service for abstracting and indexing of the chemical information. During 1973, CTR established a similar policy with the Biosciences Information Service (Biosis) in Philadelphia, and with Infodata International in Chicago, which publishes in the Index to the Periodical of the US Government. In 1980, CTR began to send advanced copies for abstracting and indexing purposes to the Franklin Institute in Philadelphia.

Office of Management and Budget Approval

In late 1979 a request for continuation of funds was submitted to the OMB. This document outlined the purpose and scope of CTR, the publication costs

incurred by the staff, a breakdown of the distribution of each issue, including the categories of subscribers, and the justification for continuing the publication. In early 1980, OMB approved continued production through January 1983. Further funding through January 1986 will be requested in mid-1982.

Distribution of CTR

The Scientific Information Branch is responsible for maintaining the courtesy mailing list for CTR. CTR is available without cost to 3225 qualified medical groups, physicians, and libraries, and is for sale to others by the Superintendent of Documents, Government Printing Office (GPO). The approximate distribution, which varies with each issue, is shown on Table 2 on the following page. In the past several years, the number of paid subscriptions has increased tremendously. The number will undoubtedly continue to rise because of the limits set for free subscriptions by the OMB each time the journal receives OMB clearance. The demand for specific back issues remains high; most requests are for the proceedings of meetings.

CANCER TREATMENT SYMPOSIA

The possibility of publishing symposia as additional issues of CTR has been explored. However, the coordinating function required to interdigitate symposia with regular issues of CTR which contain refereed manuscripts has produced extensive delays in the publication of original research and decreased the value of CTR to the scientists involved in treatment research. By initiating Cancer Treatment Symposia as a separate publication, the SIB can respond to the great demand for proceedings of scientific meetings sponsored by the Division. It will permit the timely publication of original research in the parent journal, Cancer Treatment Reports as well as the proceedings of scientific meetings. Therefore, we have requested the introduction of Cancer Treatment Symposia as a bi-monthly supplement to CTR.

THE DCT BULLETIN

The DCT Bulletin is the third publication of the Scientific Information Branch and is published four to six times annually. Each issue, which is sent to an audience of over 6000, provides information about the latest activities and programs of the Division of Cancer Treatment. Information included in the DCT Bulletin covers major policy decisions, new initiatives, and meetings sponsored by the Division. The DCT Bulletin contains progress reports about current research programs, communications on the status of new antitumor drugs that have been developed and evaluated in the Division, information on current drug availability and distribution, and announcements of research conferences, and workshops. Each issue is approximately six to eight pages in length and is accompanied by a Supplement of approximately ten to fifteen pages. The Supplement lists all recently activated clinical research protocols that are sponsored by the Division. Information contained in the DCT Bulletin assists contractors, grantees, as well as other scientists and physicians to keep abreast of new initiatives sponsored by the Division.

TABLE 2

Distribution

1. Official Use (distributed at no cost)	
a. Federal Government	
NIH employees	193
NCI contractors and grantees	840
FDA employees	17
VA employees	41
VA libraries and hospitals	79
PHS employees	10
Armed services (employees and libraries)	24
b. State agencies	9
c. Research institutes (including libraries)	551
d. Medical schools and universities (including libraries) ...	216
e. Hospitals (including libraries)	384
f. Special advisory groups	24
2. Free distribution	
a. Foreign investigators and institutions	390
b. Foreign libraries	101
c. Pharmaceutical and related industries	59
d. Individuals (such as medical practitioners)	287
3. Superintendent of Documents	
a. Paid subscriptions.....	3400
b. File copies	73
c. Depository Libraries	<u>465</u>
TOTAL	7163

LITERATURE SERVICES

The Literature Research Section was also relocated twice during the year. However, it received and filled approximately 170 requests for information. Data from the fields of chemotherapy, radiotherapy, surgery, immunotherapy, and the related chemical and biomedical disciplines are used by the staff in Decision Network review, meeting FDA requirements for IND filing, preparing clinical brochures, and as background for evaluation of toxicological and clinical studies. Responses were provided as comprehensive or selected bibliographies, computer print-outs, abstracts and copies of articles. More than half of the requests entailed manual literature searches supplemented by the various automated bibliographic retrieval systems such as Medline, Toxline, and Cancerline. Comprehensive searches with copies of articles were done for clinicians of the DCT on such subjects as the two stage surgical procedure in breast cancer, treatment of multiple myeloma, vomiting in cancer patients, and the clinical application of specific drugs. Bibliographies were prepared, or updated, for such agents as WR-2721 and other radiation protectors and sensitizers, several platinum compounds, 3-deazaguanine, AT-125, homoharringtonine, the tricyclic nucleoside, and m-AMSA. Comprehensive searches were also performed on nasopharyngeal tumor models, mutagens in urine, whole body hyperthermia, bacterial screening of metal complexes, various enzyme systems and the toxic effects of specific compounds. Although the Section no longer prepares the agent folders, bibliographies are prepared for the compounds to be discussed at Decision Network meetings. A total of 37 were compiled for five meetings.

The Section also co-ordinates NCI access to the on-line data bases of the Medlars automated bibliographic research system for all areas of the Institute, processing searches and providing assistance and instruction in the use of the system. Monthly SDI (Selective Dissemination of Information) bibliographies are produced for staff on specific subjects of continuing interest.

The Section also maintains the Cancer Therapy Library, a collection of books and journals for the use of NCI staff. Copies of over 70 journals are regularly received including abstracting and indexing secondary sources.

Collaboration with Clinical Oncology Program

Other major accomplishments in 1980-1981 include participation of the Chief, SIB, in the clinical therapeutic trials performed by the Medicine Branch, DCT, NCI. General accomplishments and publications are summarized under the report entitled Clinical Trials and Miscellaneous Clinical Investigations (Project Report 201-CM-03403-16M).

Other Publications Include:

1. Hubbard SM and Donehower MG: The nurse a in cancer research setting. Seminars in Oncology 7:9-17, 1980.
2. Hubbard SM: Effective communication through oral presentation. Oncology Nursing Forum 7:27-40, 1980.

3. Hubbard SM: Chapter 14. Chemotherapy and the Cancer Nurse. In Marino LB (Ed.), Cancer Nursing, St. Louis, The C. V. Mosby Co., pp. 287-343, 1981.
4. Johnson BL and Hubbard SM: Leukemias and Lymphomas. Chapter 7. In Jones DA, Jirovec M, and Dunbar CA (Eds.), Medical Surgical Nursing: A Conceptual Approach, 2nd Ed., New York, McGraw-Hill (in press)
5. Hubbard SM: Neoplasia, Chapter 5. In Jones DA, Jirovec M, and Dunbar CA (Eds.), Medical Surgical Nursing: A Conceptual Approach, 2nd Ed., New York, McGraw-Hill (in press)
6. Robichaud KJ and Hubbard SM: Infection and cancer. In Groenwald S (Ed.), Cancer Nursing: Principles and Practice. New York, Duxbury Press (in press)
7. Jenkins J, Hubbard SM, and Howser D: The use of intraperitoneal chemotherapy in the management of patients with ovarian cancer. Nursing 81, (in press)
8. Hubbard SM: The role of the nurse in cancer therapy and cancer treatment research. NITA, Official Journal of the National Intravenous Therapy Association July-August 1981 (in press)

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SUMMARY REPORT
ASSOCIATE DIRECTOR FOR DEVELOPMENTAL THERAPEUTICS PROGRAM
DIVISION OF CANCER TREATMENT
NATIONAL CANCER INSTITUTE

October 1, 1980 - September 30, 1981

I. Introduction

The Developmental Therapeutics Program (DTP) has primary operational responsibility for all aspects of the preclinical development of antitumor agents for the Division of Cancer Treatment (DCT). The extramural component of the DTP is located in the Blair Building in Silver Spring, Maryland, where directed drug development activities are contract-supported and research in biochemistry and pharmacology is administered through the grant mechanism. The DTP intramural laboratory operation conducts anticancer drug and other pre-clinical cancer treatment related research in Building 37 on the NIH campus in Bethesda.

The extramural program, which is devoted to the acquisition, antitumor evaluation, formulation, large-scale drug production and toxicology studies on new candidate anticancer drugs, is managed by six Branches: Drug Synthesis and Chemistry, Natural Products, Drug Evaluation, Animal Genetics and Production, Pharmaceutical Resources, and Toxicology. The Extramural Research and Resources Branch is responsible for the management of cancer-related biochemistry and pharmacology grants. An eighth Branch, the Information Technology Branch, is being formed to manage the extensive DTP computer-related information management requirements.

The intramural program is conducted through four Laboratories: Medicinal Chemistry and Biology, Molecular Pharmacology, Tumor Cell Biology and Chemical Pharmacology. Intramural research is supportive of both new drug studies and basic investigations in cancer-related biochemical processes and molecular biology.

The Office of the Associate Director is responsible for the leadership and management of the Developmental Therapeutics Program and the accomplishment of the goals and objectives of the DCT pre-clinical program.

II. Accomplishments

A. Extramural Program

1. Acquisition of new materials as potential anticancer drugs

a) Drug Synthesis and Chemistry Branch (DS&CB)

The procurement of synthetic compounds for screening continues to involve an increasing degree of intellectual selection. Over

25,000 structures were evaluated as potential new agents by both computer methods and human inspection. Selective acquisition of 12,900 of these materials using predictive techniques resulted in a yield of 5.6% P388 leukemia actives. This compares with last year's 4.4% yield when the computer technique was available for only part of the year. An additional computer program called Automated Group Analysis and Projection (AGAP), has recently been designed and installed by the DS&CB. This analysis process should allow even more sophisticated analyses of the relationship between chemical structure and antitumor activity.

Contracts for the directed synthesis of radiosensitizers, nucleosides which inhibit key enzyme systems, and natural product analogs produce compounds for evaluation as potential antitumor agents. In addition, the task order contract mechanism was utilized successfully to obtain 200 specially selected compounds for biological evaluation.

A significant recent achievement has been the development of an interlink between the DTP chemical structure and biological data files. It is now possible, using an appropriate CRT terminal, to enter an NSC number and retrieve structure, inventory and summarized antitumor test data. Upon completion of additional programming, the interlink will become searchable as well as displayable and an even more powerfully interactive structure-activity tool will be available.

b) Natural Products Branch (NPB)

Natural products continue to play a major role in the DTP compound acquisition process. Prescreens are important in identifying the crude fermentation products which should be investigated further. Using prescreens, a P388 in vivo activity rate of 15% was achieved. This is far greater than the 2-3% rate which might be expected from the random screening of materials. In addition to the normal screening of fermentation products, two contractors are investigating the possibility that microbial biotransformation and co-metabolism processes may convert known antitumor agents into even more useful drugs.

The initial step in the plant product program is the collection of plant samples from various parts of the world by the USDA. Extracts which are active trigger the re-collection of larger plant samples which are extracted and assigned to plant fractionation chemists who isolate the active components. Including compounds obtained from non-contractual sources, 232 pure plant and animal products were obtained for evaluation.

A marine animal product, didemnin B, passed DN2A in the NCI Linear Array. Three natural products, taxol, homoharringtonine and echinomycin, passed DN2B and were entered into toxicology.

2. Biological evaluation

a) Animal Genetics and Production Branch (AG&PB)

The recently established AG&PB provides healthy animals with properly defined genetic characteristics for the large-scale testing done by DTP and certain other parts of NCI, NIH, and the Federal Government. The majority of the animals provided are mice, but significant numbers of dogs are also required for toxicology protocol testing. Both breeding and diagnostic contracts are utilized to produce and monitor the health and genetic characteristics of the animals required. Recent advances in technology now permit the AG&PB to provide "super clean" animals to those facilities able to maintain a disease-free environment. Animals of this type are required for tests using human tumor xenografts and for use with biological response modifiers.

b) Drug Evaluation Branch (DEB)

During the period 4/80-3/81, over 22,000 materials (13,800 synthetics, 8,700 crude natural products) were screened in vivo for the first time. A total of 1,800 compounds have been selected for the tumor panel over the past few years with 670 having complete panel test data as of 3/81. New compounds are being entered into the panel at the rate of about 250 per year and being completed (all eight tumor models finished) at about 400 per year. The DTP goal is to obtain complete data on 2,000 compounds. Given the number of compounds completed, the number partially completed, and the rates mentioned above, an adequate number should have been entered into the panel by the end of 1982 and complete test data could be available on 2,000 compounds by the end of 1983. While this time period could be shortened somewhat by increasing the panel test rate, it would have to be accomplished at the expense of our P388 leukemia prescreen for new materials, given constant resources.

Some preliminary answers to questions addressed by the panel are beginning to emerge. The solid tumors in the panel are selecting compounds which would have been missed by L1210 leukemia alone. Human tumor xenografts are selecting some compounds which would not have been selected by murine tumors. There is little correlation between human xenograft and mouse tumors of the same type (e.g. colon) in responsiveness to drugs.

A new in vitro screening initiative has been undertaken with the advent of testing in the human tumor stem cell cloning assay (HTSCCA). The first phase of this new screening technique, which utilizes fresh human tumor specimens grown in cell culture, is a blind study of 50 drugs by four contractors. When this study is completed in late 1981 and the data are analyzed, we will begin to screen 1,000 new acquisitions per year in this assay.

A number of new tumor models are being developed under several contracts. Of particular interest is the finding that fresh human surgical specimens can be grown under the renal capsule and drug response evaluated in mice. Moreover, immunocompetent mice may be used. In a retrospective study, this technique predicted 13 of 15 positive clinical responses and 5 of 5 failures. In a prospective study, utilizing therapy predicted as effective by this model, 5 of 10 responses were observed.

3. Formulation and bulk chemical procurement

The Pharmaceutical Resources Branch (PRB) conducts a program of large-scale synthesis, procurement and formulation of materials of interest to the DTP. Over 400 kilograms of synthetic materials were prepared for formulation into investigational clinical products. Among these large-scale projects were the synthesis of PALA, methyl-GAG, desmethylnisonidazole and dihydro-5-azacytidine. The required amounts of 128 compounds destined for tumor panel testing were also prepared. Twenty-four radiolabeled substances were procured and characterized through synthesis and purchase in support of antitumor agent pharmacology studies.

A number of difficult analytical and formulation problems were resolved in an area of continuing concern, i.e. poorly water-soluble compounds designated by the Decision Network Committee as potential clinical agents. The recent development of a complex vehicle delivery system for spirohydantoin mustard illustrates a success of this type. Formulation contractors produced almost 600,000 parenteral doses of investigational antitumor drugs for Program use. The intramural laboratory component of the PRB was assigned several of the more difficult new agent formulation and analytical problems. Progress was achieved in several problem areas related to natural products (taxol, THC, ellipticine) and synthetics (Pt analogs).

4. Preclinical toxicology

The Toxicology Branch (TB) is using a new toxicology protocol, which was developed in collaboration with the FDA. The protocol has been utilized to initiate ten studies with several additional studies anticipated prior to the completion of the reporting period. The ten new agents already assigned to full Task I toxicity protocols are N-methylformamide, ellipticine, homoharringtonine, spirohydantoin mustard, CBDCA, dihydro-5-azacytidine, tricyclic nucleotide, Henkel compound, echinomycin and a discreet compound.

In addition to the mouse and dog studies required by the FDA, the NCI is conducting additional mouse toxicity studies in order to determine whether the mouse alone would be a satisfactory qualitative and quantitative predictor of human clinical toxicity. A retrospective study with 20 established oncolytic agents also is underway to evaluate the mouse as a predictive model.

5. Grants in pharmacology and biochemistry

The newly created Extramural Research and Resources Branch (ERRB) provides scientific guidance for extramural projects mainly through the grants mechanism. This branch supported 358 grant projects representing over 35 million dollars in direct costs. Projects of NCI interest, with special relevance to the drug discovery and other objectives of the DCT, are administered. These grants deal mainly with the following antitumor agent areas: synthesis and chemistry, natural products, experimental therapeutics, pharmacology and mechanism of action.

6. Summary of Extramural Drug Development Accomplishments

The past year has proved to be an active and productive period. Twenty-five thousand synthetic chemical structures were conducted as potential new antitumor agents, 12,900 of these were selectively acquired and 5.6%, or 722, were active against leukemia P388 in vivo. A total of 5,382 new extracts of natural origin was obtained for screening in addition to 424 pure compounds. Almost 12,000 fermentation experiments yielded 1,734 materials which were active in one or more in vitro prescreens. From this group, 950 were tested in vivo with 15% found to be active. During the year, 160 active natural product extracts were chemically fractionated and 18 new active natural products were identified.

In screening, 22,500 materials were screened in vivo for the first time and 1,800 compounds were assigned to the tumor panel. New compounds enter the panel at a rate of 250 per year and approximately 400 complete the panel each year. As of March, 1981, 670 compounds have completed panel testing and it is now projected that the desired 2,000 compounds could complete testing by the end of 1983.

Also prepared during the year were 400 kg of synthetic materials, 24 radioactively labeled drugs, and 600,000 parenteral doses of agents for clinical use. Ten new Task I toxicology studies were initiated under the new protocol.

Sixteen compounds passed DN2A, 6 compounds passed DN2B, and 3 compounds passed DN3.

B. Intramural Program

1. Laboratory of Chemical Pharmacology (LCP)

Both misonidazole and its metabolite, desmethylnisonidazole, are effective hypoxic cell sensitizers. The relationship of this metabolic step to the neurotoxicity observed as the dose-limiting clinical toxicity is unknown. As part of a study in this area, the O-demethylation of misonidazole was shown to be mediated by a cytochrome P450 mixed function oxidase.

Studies on the pharmacology and mechanism of action of m-AMSA have continued during the past year. Using rat liver microsomes, it was found that the metabolism of m-AMSA is optimal in a system comprised of microsomes, cytosol, oxygen and an NADPH generating system. HPLC analysis revealed that rat liver microsomes incubated with m-AMSA form two products: N'-methanesulfonyl-N-(9-acridinyl)-3'-methoxy-2', 5'-cyclohexadiene-1', 4'-diimine (m-AQDI) and 3-methoxy-4'-(9-acridinylamino)-2', 5'-cyclohexadien-1'-one (m-AQI). m-AQDI can be formed via an N-hydroxylated intermediate and the metabolism of m-AMSA is postulated to proceed via this route, resulting in bioactivation to a highly reactive species. An in vitro cell colony assay in soft agar utilizing L1210 cells was established to determine the relative cytotoxicities of m-AMSA, m-AQDI and m-AQI. m-AQDI and m-AQI are approximately 100-fold more toxic than m-AMSA and, unlike m-AMSA, toxicity is not time-dependent. The principal in vivo biliary metabolite of m-AMSA, m-AMSA-5-glutathione, was not toxic. Derivatives of m-AMSA were synthesized in which the nitrogen of the anilino ring was substituted to form a tertiary amine. These derivatives were non-toxic, supporting the hypothesis that N-hydroxylation is the initial reaction in the metabolism of m-AMSA. These results indicate that m-AMSA is bioactivated with time in vivo to a highly reactive species capable of reacting with critical macromolecules causing cell death, or with molecules such as glutathione resulting in detoxification.

The possibility of "opening" the blood-brain barrier (BBB) by the inhalation of 20% CO₂ for 2 hours or more was investigated by multiple isotope detection. This treatment had no discernible effect on the blood-brain K_i's of two substances, the moderately permeable ion, ⁴²K, and the slightly permeable amino acid, AIB; however the transfer across the BBB of DTPA, a virtually impermeable compound, was increased 5-20 fold by the exposure of high blood levels of CO₂. This peculiar change in BBB permeability suggests that CO₂ inhalation may enhance the exchangeability of very impermeable drugs between blood and brain.

The ability of liposomal encapsulation of antitumor agents to alter their disposition, lymphatic uptake, and therapeutic effects against experimental lymph node metastasis has also been evaluated during the past year. Liposomal encapsulation also altered the peritoneal absorption, tissue distribution, metabolism and excretion of ara-C. Liposome entrapment of ara-C reduced the rates at which the drug was absorbed from the peritoneal cavity and excreted in urine while enhancing lymphatic uptake of the drug by more than 10-fold. Most striking was the localization of ¹⁴C-activity in renal and thoracic lymph nodes of rats given liposome entrapped ara-C, with 300-1000 fold higher levels present than in corresponding lymph nodes of rats receiving the free drug. The metabolite conversion of ara-C to uracil-β-D-arabinofuranoside was reduced by approximately 3-fold following liposome entrapment of the drug. The enhanced lymphatic uptake and the localization and persistence of adriamycin and ara-C

in lymph nodes, resulting from liposome entrapment of the drugs, may be of benefit in treating tumors that metastasize via lymphatic pathways.

Cells that contain uridine/cytidine kinase have the ability to utilize extracellular uridine in order to circumvent inhibition of the de novo pyrimidine biosynthetic pathway. The ability of uridine at normal serum levels for humans, rats, and mice (2-12 μ M) to reverse the cell growth inhibitory effects of PALA, an inhibitor of de novo pyrimidine biosynthesis, was determined in tissue culture. Since both L1210 cells and Lewis lung carcinoma cells rapidly deplete uridine from cell culture media, a method for infusing uridine was devised in order to maintain the media uridine at constant levels. Extracellular uridine at normal serum levels was found capable of markedly reducing the growth inhibitory effects of PALA on both cell lines. Thus, serum uridine may reduce the antitumor effectiveness of inhibitors of the de novo pyrimidine biosynthetic pathway in vivo. Three such inhibitors were studied to determine their effects on circulating uridine concentrations in BDF₁ mice. Pyrazofurin and 6-azauridine had no significant effects on serum uridine levels, whereas PALA reduced serum uridine levels by 55%. This reduction could contribute to the antitumor effectiveness of PALA by limiting the rescue of cells possessing a salvage pathway. Although serum uridine levels in patients treated with PALA were also found to decrease from predose serum levels, they generally remained in the range of serum levels for normal humans. However, mouse serum uridine levels after PALA treatment consistently fell to about half of the normal range which may help to explain, in part, why PALA is curative towards Lewis lung carcinoma cells in BDF₁ mice but is ineffective in humans.

A study was initiated to examine the relative use of de novo pyrimidine biosynthesis versus salvage of pyrimidine nucleotides by cells in tissue culture. The incorporation of ¹⁴C-bicarbonate into uracil nucleosides of L1210 cells was determined under conditions in which the uridine concentration in the medium was maintained constant. The results indicate that at normal serum levels, L1210 cells depend mainly on extracellular pyrimidines rather than newly synthesized pyrimidines to maintain their pyrimidine pools. If this is also the case for tumor cells in vivo, then the lack of therapeutic response by humans towards inhibitors of de novo pyrimidine biosynthesis may be explained by a reduced dependency of tumor cells on the de novo pyrimidine biosynthetic pathway.

The effects of inducers of cellular differentiation, such as DMSO and BA on membrane microviscosity, have been determined by ESR spectroscopy. Friend erythroleukemia cells that had been differentiated by both DMSO and BA showed marked decreases in membrane microviscosity. Preliminary data indicate that microviscosity changes are not a steady transition from control levels to differentiated levels; instead, a decrease in microviscosity appears to occur during the first 24 hours of exposure to the inducing agent after which progressive increases in microviscosity are seen.

The toxicity, as well as the therapeutic properties, of agents used to treat cancer has been evaluated during the past year. Utilizing a rat myocyte system developed in this laboratory, the cardiotoxic potential of several anthracyclines, two anthracenediones, and m-AMSA has been tested. m-AMSA is toxic to the beating cells in a manner very similar to that of adriamycin, but the anthracenediones were not toxic at the limits of their solubilities (greater than 10^{-2} M). Two anthracyclines, AD-32 and aclacinomycin A, were not toxic at the maximal concentrations tested. They were also the only two anthracyclines evaluated that did not appear to bind to nuclei of the heart cells as evaluated in viable cells with the fluorescence microscope.

The availability of a large breeding and experimental colony of non-human primates (500 animals representing 4 species) continues to provide the LCP and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, biochemical and carcinogenesis studies. The carcinogenic effects of 27 substances, including antineoplastic and immunosuppressive agents, model rodent carcinogens, pesticides, artificial sweeteners and contaminants of human foodstuffs are currently being evaluated. Eight of the 27 substances evaluated have induced malignant neoplasms in nonhuman primates, producing a tumor incidence ranging from 9.1-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B₁, methyl-azoxymethanol acetate, procarbazine, methylnitrosourea, and urethane. In addition, single cases of malignant tumors have been diagnosed in animals treated with adriamycin, N-methyl-N'-nitro-N-nitrosoguanidine and N,N'-dimethyl-p-phenylazoaniline (butter yellow).

2. Laboratory of Medicinal Chemistry and Biology (LMCB)

The LMCB has an interest both in new antitumor agents originating from the scientific community as a whole, and also in agents originating within the LMCB. Of the latter group, the compound AZQ (NSC-182986) is presently in Phase II clinical trial, spirohydantoin mustard (NSC-172112) is undergoing toxicology evaluation, and a Phase I clinical trial will likely be initiated with dihydro-5-azacytidine (NSC-264880) during the current fiscal year.

A HPLC assay for AZQ was used to measure the drug in the plasma, urine and CSF of 20 patients receiving dose-levels ranging from 1-20 mg/m² during a Phase I trial conducted in the Medicine Branch, DCT. All subjects showed a very rapid redistribution phase ($t_{1/2}(\alpha)$ = 2.8 ± 1.3 min) followed by a slower yet still fast plasma elimination phase. The mean elimination half-life for the terminal phase of the two-compartment open model to which the data was fitted was 33.3 ± 4.5 min and was dose independent. The pharmacokinetics in man closely paralleled those in the rat, dog, and rhesus monkey. A mean total body clearance of 517 ± 155 ml/min implied the involvement of hepatic as well as renal clearance.

The urinary excretion of unchanged AZQ was examined in 5 patients receiving 10 mg/m² or more of the drug. Measurable amounts of intact drug could be found in only two of these patients and represented less than 0.2% of the total administered dose. Cerebrospinal fluid, obtained via lumbar puncture, was available from 3 different patients, including one from two different dosage cycles. AZQ was found to enter the CSF, and to attain concentrations that were substantial compared to plasma levels. Detailed studies of AZQ metabolism and pharmacokinetics will continue during Phase II clinical trials of the drug.

Considerable progress has been made in efforts to prepare cytidine deaminase inhibitors for potential use in combination with antitumor agents such as ara-C whose clinical utility is reduced by enzyme-catalyzed deamination in vivo. In the ring-expanded pyrimidine nucleoside series, three compounds have been prepared with K_i values in the range 1 to 5 x 10⁻⁸M, i.e., approximately one order of magnitude more active than the reference compound tetrahydrouridine; the latter agent was the most active cytidine deaminase inhibitor described prior to the present studies, and the only compound of this type which has entered clinical trial.

Efforts have continued in the design and synthesis of amino acid analogues which are activated to cytotoxic compounds by the enzyme tyrosinase, and which could therefore be anticipated to show activity against melanotic melanoma, a tumor with high levels of this enzyme. Of the three initial target compounds, two are readily oxidized substrates for the enzyme, and also exhibit inhibitory activity against melanotic B16 melanoma in vitro, with ID₅₀-values of 4.5 x 10⁻⁶M and 7.5 x 10⁻⁶M.

Studies indicated that the synergistic effect of the combination of PALA and 5-FU on the growth of MDA cells correlates with an increased proportion of 5-FUTP in the pyrimidine nucleotide pool, and consequently with an enhanced incorporation of 5-FU into RNA, but not with inhibition of DNA synthesis.

The lethal and sublethal effects of sangivamycin were studied in sarcoma 180 in vitro in relation to drug concentration and duration of drug exposure. Sangivamycin lethality was found to be dependent on both drug concentration and duration of drug exposure. Pronounced effects on cell survival were observed only when sangivamycin exposure was prolonged; with prolonged drug exposure, small increments in sangivamycin concentration resulted in large increases in cell killing. Log phase cells were more susceptible to the lethal effects of sangivamycin than early plateau phase cells. Measurements of incorporation of [³H]thymidine and [³H]uridine into the acid-insoluble cell fraction demonstrated inhibition of both DNA and RNA synthesis by sangivamycin which was also dependent on drug concentration and duration of drug exposure, reflecting the lethality characteristics of sangivamycin. These findings indicate that maximum lethality is obtained by prolongation of sangivamycin

exposure, and suggest that pharmacokinetic studies may be important for determining regimens which provide such exposure in man.

Interest in the L-glutamine antagonist DON [6-diazo-5-oxo-L-norleucine (NSC-7365)], was renewed as a consequence of the finding of its effectiveness in treating human lung and colon xenografts in athymic mice. Analysis of acid-soluble nucleotide pools after treatment with DON indicated a reduction in the concentration both of adenine and guanine nucleotides. Furthermore, the synthesis of RNA and DNA by cells in culture was potently inhibited, whereas protein synthesis was not affected. A study of the ability of purines, pyrimidines and amino acids to reverse the cytotoxicity of DON to P388 cells revealed that adenine, adenosine, hypoxanthine and L-glutamine were effective counteragents. These studies confirm that DON behaves principally as an inhibitor of the de novo purine biosynthetic pathway.

Several studies related to the toxicity of bleomycin have shown that purified NADPH cytochrome P450 reductase mediates bleomycin-DNA chain breakage and that reactive oxygen is involved in bleomycin mediated DNA cleavage in biological systems. The chemiluminescence, which occurs when bleomycin reacts with lung microsomes, is being utilized as the basis for a model system to help understand both the therapeutic utility and toxic properties in lung cells.

3. Laboratory of Molecular Pharmacology (LMP)

Previous work in the LMP had shown that a particular DNA repair defect affecting removal of O⁶-alkyl-guanine lesions confers sensitivity of cells to chloroethylnitrosoureas. Some human tumor cells have this defect, whereas others do not. Repair-defective cells (designated Mer⁻ phenotype) treated with chloroethylnitrosoureas, produce increased amounts of interstrand crosslinks. This repair defect was found to be specific for nitrosoureas and did not govern interstrand crosslinking by other classes of drugs, such as cis-platinum or nitrogen mustards. The implication is that the susceptibility of a given strain of tumor cells to a particular type of DNA-damaging drug may depend on the presence of a particular DNA repair defect. Attempts are being made to identify other DNA repair deficiencies in human tumor cell strains, and, if such are found, to identify DNA-damaging drugs that could take advantage of this vulnerability.

A fluorometric method was devised to permit DNA alkaline elution assays without the use of radioactive DNA labeling. With the use of these techniques, it was possible to show that, contrary to reports from other laboratories, the chemically induced in vitro differentiation of erythroid or myeloid leukemia cells is not accompanied by the production of DNA strand breaks.

The new histone variants, designated H2A.X and H2A.Z, were characterized in terms of peptides which they contain in common with the predominant H2A species. Improvements in the separation techniques for phosphorylated and acetylated histones were devised and used to determine the types of modifications which the new histone species undergo. A finding of importance is that the amino acid sequence of H2A.Z is highly conserved in phylogenically diverse organisms. This applies to the non-H2A-like part of the sequence, which is even more highly conserved than H2A itself. This indicates that H2A.Z has a highly specific function.

A recent, major discovery was that, contrary to the common histone species whose synthesis is restricted to the DNA synthesis phase of the cell cycle, the new variants H2A.X and H2A.Z are synthesized at other phases of the cell cycle. This basal synthesis of histones also include the variant H3.3 and the normal H2B and H4 species, but the main H2A species are not made. Inhibition of DNA synthesis with hydroxyurea inhibited the synthesis of the common H2A species while the synthesis of H2A.X and H2A.Z continued.

4. Laboratory of Tumor Cell Biology (LTCB)

Two new human virus isolates called human T cell lymphoma (leukemia) virus strains CR and MB (HTLV_{CR}, HTLV_{MB}) have been extensively characterized. They are not significantly related to any known animal retrovirus. These are the first unambiguous human retrovirus isolates.

Infectivity of the newly isolated retrovirus from a patient with cutaneous T cell lymphoma (HTLV) was confirmed by in vitro transmission of the virus to T cells of some normal relatives of a patient with acute lymphocytic leukemia. Primate retroviruses and HTLV have been shown to induce a new antigen (human activated antigen or HAA). This antigen may be related to T cell growth factor (TCGF) receptor. Solid phase radioimmunoassay has been developed to detect natural antibodies to HTLV proteins in sera of patients with T cell malignancies. This is the first evidence of a specific immune response in humans to a retrovirus. Serological studies also show the presence of natural antibodies to internal proteins of HTLV in sera of patients with cutaneous T cell leukemias and lymphoma. These studies suggest that HTLV is an acquired virus.

T cell growth factor (TCGF) has been purified to near homogeneity from PHA stimulated lymphocyte conditioned media. TCGF was isolated from the surface of cells from patients with T cell leukemias. This growth factor has been shown to be different from TCGF obtained by lectin stimulation of normal peripheral blood lymphocytes. In vitro translation product of mRNA isolated from TCGF producing cells has been shown to be biologically active.

Retinoic acid has been shown to induce terminal differentiation of myeloid cells from patients with acute promyelocytic leukemia.

Induction of a histone polypeptide (HP) has been observed in acute promyelocytic cells (HL60) after differentiation with dimethyl sulfoxide. It can be potentially useful as a marker of differentiation. The HP has been found to be specific for human acute leukemias. HP was not detected in leukocytes from patients with chronic leukemias.

Molecular cloning and analysis of the first human 'onc' genes, i.e., the human cellular homologue of the sarc gene of simian (v-sis) and feline (v-fes) sarcoma virus has been accomplished.

Molecular cloning of the genomes of simian sarcoma virus and the associated helper virus has been accomplished. Transformation specific sequences (v-sis) of simian sarcoma virus, as a distinct viral onc gene, have been identified. Technology for the sequencing of DNA has been developed. Partial sequence of the env gene and onc gene region of simian sarcoma virus has been determined.

TABLE I

COMPOUNDS THAT PASSED DECISION NETWORK 2A, 2B, & 3 (4/01/80 - 3/31/81)

<u>NSC#</u>	<u>NAME</u>	<u>TYPE</u>
2A		
65346	Sangivamycin; 7H-Pyrrolo[2,3-d]pyrimidine-5-carboxamide, 4-amino-7-β-D-ribofuranosyl-	NP
146068	Ed Malonato; Platinum, (1,2-ethanediamine-N,N') [propanedioato(2-)-0,0']-, (SP-4-2)-	S
233853	Adriamycin octanoylhydrazone; Octanoic acid, [1-[4-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]-1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-2-naphthacenyl]-2-hydroxyethylidene] hydrazide, monohydrochloride (2S-cis)-	SS
241240	CBDCA; Platinum, diamine[1,1-cyclobutanedicarboxylato(2-)-0,0']-, (SP-4-2)-	S
256927	Chip; Platinum, dichlorodihydroxybis(2-propanamine)-, (OC-6-33)-	S
266210	Bisdaunorubicinhydrazone; Butanedioic acid, bis[[1-[4-[(3-amino-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl)oxy]1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy-7-methoxy-6,11-dioxo-2-naphthacenyl]-ethylidene]hydrazide], dihydrochloride, stereoisomer	SS
267702	Cyclohexyl pyrrolizine derivative, Carbamic acid, cyclohexyl-, [5-(3,4-dichlorophenyl)-2,3-dihydro-1H-pyrrolizine-6,7-diyl] bis(methylene)ester	S
268242	N-Dibenzyl-daunorubicin; 5,12-Naphthacenedione,8-acetyl-10-[[3-bis-(phenylmethyl)amino]-2,3,6-trideoxy-α-L-lyxo-hexopyranosyl]oxy]-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy-, hydrochloride, (8S-cis)-	SS
269148	7-Omen; 2,6-Epoxy-2H-naphthaceno[1,2-b]oxocin-9,16-dione, 4-(dimethylamino)-3,4,5,6,11,12,13,14-octahydro-3,5,8,10,13-pentahydroxy-11-methoxy-6,13-dimethyl-	SS
271674	DACH; Platinate(1-), [1,2,4-benzenetricarboxylato(3-)-0 ¹ ,0 ²](1,2-cyclohexanediamine-N,N')-, hydrogen, (SP-4-3)-	S
276382	Discreet	NP

<u>NSC#</u>	<u>NAME</u>	<u>TYPE</u>
2A		
284356	Gulf Oil Compound; 4,8-Ethenopyrrolo[3',4':3,4]cyclobut [1,2- <u>f</u>]isoindole-1,3,5,7(2 <u>H</u> ,6 <u>H</u>)-tetrone, octahydro-	S
298223	CC 1065; Benzo[1,2- <u>b</u> :4,3- <u>b'</u>]dipyrrole-3(2 <u>H</u>)-carboxamide, 7-[[1,6-dihydro-4-hydroxy-5-methoxy-7-[4,5,8,8a- tetrahydro-7-methyl-4-oxocyclopropa[<u>c</u>]pyrrolo[3,2- <u>e</u>]indol- 2(1 <u>H</u>)-yl]carbonyl]benzo[1,2- <u>b</u> :4,3- <u>b'</u>]dipyrrol-3(2 <u>H</u>)-y] carbonyl]-1,6-dihydro-4-hydroxy-5-methoxy-	NP
305884	Acetamide, <u>N</u> -methyl- <u>N</u> -[4-[(7-methyl-1 <u>H</u> -imidazo[4,5- <u>f</u>] quinolin-9-yl)amino]phenyl]-, monohydrochloride	S
325319	Cyclic depsipeptide; Leucine, 1-(2-hydroxy-1-oxopropyl) prolyl- <u>N</u> -methylleucylthreonyl-4-amino-3-hydroxy-6- methylheptanoyl-4-hydroxy-2,5-dimethyl-3-oxohexanoyl- <u>N</u> , <u>O</u> -dimethyltyrosylprol-yl-, ϕ -lactone	NP
2B		
3051	Formamide, <u>N</u> -methyl-	S
125973	Taxol; Benzenepropanoic acid, β -(benzoylamino)- α -hydroxy-, 6,12b-bis(acetyloxy)-12-benzoyloxy)-2a,3,4,4a,5,6,9,10,11, 12,12a,12b-dodecahydro-4,11-dihydroxy-4a,8,13,13-tetramethyl- 5-oxo-7,11-methano-1 <u>H</u> -cyclodeca[3,4]benz[1,2- <u>b</u>]oxet-9-yl ester, [2a <u>R</u> -[2a α ,4 β ,4a β ,6 β ,9 α (α <u>R</u> *, β <u>S</u> *),11 α ,12 α ,12a α ,12b α]]-	NP
280594	Tricyclic nucleoside, phosphate salt; 1,4,5,6,8- Pentaazaacenaphthylen-3-amine, 1,5-dihydro-5-methyl-1- (5- <u>O</u> -phosphono- β - <u>D</u> -ribofuranosyl)-	S
286193D	Discreet	S
296961	WR-2721; Ethanethiol, 2-[(3-aminopropyl)amino]-, dihydrogen phosphate (ester)	S
312887	9 <u>H</u> -Purin-6-amine, 2-fluoro-9-(5- <u>O</u> -phosphono- β - <u>D</u> arabinofuranosyl)-	S
526417	Quinomycin A(Echinomycin)	NP
3		
261036	RO 5-9963; 1,2-Propanediol, 3-(2-nitro-1 <u>H</u> -imidazol-1-yl)-	S

S = synthetic

SS = natural product modified synthetically

NP = natural product

ANNUAL REPORT OF THE DRUG SYNTHESIS & CHEMISTRY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 - September 30, 1981

The Drug Synthesis and Chemistry Branch (DS&CB) occupies the front-end position in the Linear Array for drug development. As such, the fundamental responsibility of the Branch is the acquisition and management of the flow of unique synthetic compounds for evaluation as potential anti-cancer agents in the Developmental Therapeutics Program (DTP).

The DS&CB achieves its central mission by engaging in a variety of Program activities, namely; selective acquisition, Task Order syntheses, contract syntheses, Cancer Research Emphasis Grants (CREGS), storage and distribution, Chemical Information System (CIS) and worldwide surveillance.

The DS&CB has undergone major shifts both in program and personnel. Consistent with the Division of Cancer Treatment's (DCT) policy, the branch has phased out several research contracts as well as CREGS. We have fully implemented the new Task Order contract mechanism for the synthesis of compounds of interest. Working closely with the Drug Evaluation Branch, we have accomplished the initial chemistry-biology interlink. The Program staff has assumed new roles and responsibilities as dictated by the dynamics of a changing Program.

Objectives

The objectives of the DS&CB are: to acquire unique chemical agents for evaluation as potential antitumor agents; to explore and develop new classes of chemicals as preclinical candidates; to develop and utilize the CIS; to manage the accession, storage and distribution of all the chemical agents that enter the Program; to maintain worldwide surveillance to ensure a steady flow of novel compounds to the Program, and to disseminate developing structure-activity relationships to the scientific community.

Program Organization

To meet the above objectives, the DS&CB is organized on traditional lines, namely, the Office of the Chief, the Drug Synthesis Section, and the Chemical and Drug Information Section. Presently the Branch is staffed by eight professionals and five technical and clerical personnel. The Section Head vacancies have not been filled as yet. Overlaying the traditional hierarchy, the branch has adopted a matrix organizational structure wherein the branch staff is assigned tasks depending on the changing needs of the Program and on the expertise of the individual staff.

I. The Drug Synthesis Section

The objectives of the Drug Synthesis Section include the acquisition of novel synthetic compounds for antitumor evaluation and other activities related to the rapid and timely progression of compounds through the Linear Array. Novel compounds are acquired through Task Order syntheses, contract programs and acquisitions from the scientific community. The Section works in close cooperation with the various analog committees to design and develop improved analogs of existing drugs and to explore new classes of chemicals as potential preclinical candidates. The choice of compounds for synthesis is based on chemical, physical and biological rationale (including biochemical, toxicological, pharmacological, and clinical information), screening leads, etc. The Section is responsible for the technical and administrative management of CREGS*. In addition, the Section makes recommendations on the Program relevance of grants.

II. Chemical and Drug Information Section

The objective of the Section is to develop and operate a data processing system and to devise and use various computerized techniques to meet the scientific, operational control and management planning needs of the branch. The Section is concerned with: (1) operating, maintaining, and improving the automated CIS which supports the accessioning, storage and distribution of chemicals and drugs acquired for antitumor testing; (2) managing the storage and retrieval of chemical information in both automated and manual DTP files, and (3) developing methods to interlink the chemistry and biology files.

III. Acquisition of Chemicals

The objectives of this effort are to insure the continuous flow of approximately 13,000 selected synthetic compounds annually for the primary screen. Extensive contacts are maintained with industry, government laboratories, and the scientific public in academic and research institutions. Effort is directed toward (1) developing and maintaining sources for acquiring compounds with unique structural features and biological activity; (2) maintaining an effective selective ratio of inputs of compounds; (3) the acquisition of compounds for the primary screen and Tumor Panel evaluations; (4) developing and implementing selection criteria; (5) developing computer models to facilitate selective acquisition; (6) conducting structure-activity correlation studies, and (7) monitoring the discreet agreements with industrial suppliers.

In addition, the branch works closely with DEB for the selection of compounds for the Tumor Panel and shares with the Chemical Resources Section of the Pharmaceutical Resources Branch (PRB) the responsibility to acquire approximately 300 Tumor Panel compounds annually.

*See Table 1

IV. Chemical Storage and Distribution

DS&CB has the responsibility for the storage, distribution, and inventory of chemicals, bulk drugs, and crystalline natural products - activities that are basic to the operations of every segment of DTP. Operating through the contractor, the DS&CB packages and ships approximately 1300 compounds a month to research laboratories throughout the world. This requires the contractor to establish and maintain an effective working interface with other contractors and branches of DTP. Currently, about 370,000 compounds are in storage.

V. Worldwide Surveillance

The branch continues to develop and maintain extensive contacts with the chemical and pharmaceutical industries, grantees, academic institutions, research organizations and other government laboratories on a worldwide basis. Every member of the staff participates in this activity. Since this activity is essential for the achievement of the objectives for the branch, we have initiated additional contract support in this area which became operational in FY 81. A well coordinated, timely intelligence gathering is essential for ensuring the flow of unique compounds vital to the Program.

Program Operation

I. Selective Acquisitions

- A. Selective acquisition efforts occupy a central role in the activities of DS&CB. The past year has been extremely productive. Four discreet compounds, NSC's 253272D, 286193D, 305884D, and 327471D were assigned to DN₂ (Table 2). NSC-286193D the thiazole-C-nucleoside, acquired from ICN, is remarkably active against Lewis Lung carcinoma in mice producing 10 out of 10 long-term survivors at several dose levels. It has also demonstrated good activity against L-1210 and P-388 murine leukemias in vivo. NSC-286193D is identified as a high priority candidate for clinical evaluation.

During this period, several operational procedures were streamlined leading to increased efficiency in procuring both refill samples for confirmatory testing as well as compounds selected for tumor panel testing.

The highlights of the acquisition activities are summarized below:

- ° 25,083 chemical structures were acquired and processed through the preselection process.
- ° 13,886 compounds were selected as potential candidates for acquisition.
- ° 12,900 compounds were acquired, assigned NSC numbers and processed.

° Based on projections, utilizing the computational model developed by DS&CB (AGAP), the percentage of P-388 actives is estimated to be 5.6% this year as contrasted to 4.4% in 1979.

This reporting period represents the first full year in which computer-generated scores played a role in the preselection of compounds for acquisition. In 1979, approximately 4,500 compounds were rated by the Hodes Model to observe how it performed in practice. The results were suggestive of partial success. There was some enrichment in the number of actives obtained, about the same enrichment obtained by chemist review, and it was statistically significant (Hodes, Computer-Aided Selection of Compounds for Activator Screening: Validation of a Statistical Heuristic Method, Chemical Information and Computer Sciences, in press). Even though the enrichment was equal, the chemist and the computer selected somewhat different compounds. A decision was made to use the computer scores in conjunction with chemist review, and to follow the results closely by AGAP analysis (see below).

The screening results obtained for the first year using Hodes' scores are not yet complete to draw a valid conclusion. However, AGAP projections are consistent with the previous test results. The Hodes' scores used in conjunction with chemist review yields an enrichment similar to chemist review alone. It is too early to assess the impacts of the change to a QD1-5 injection schedule. DS&CB will continue to seek improvements in the Hodes' Model. The observation of fluctuations in the yield of actives as scoring ranges progress upward, suggested changes in the data set used to "train" the Model. An experiment involving a redesigned training set was initiated in the Fall of 1980 and is in progress. Further details of Hodes' Model are presented elsewhere in this report.

AGAP is a new Automated Group Analysis and Projection program designed by DS&CB to meet its acquisition demands. It is a refinement of the Cri Code analysis described in last year's report, but it is considerably more sophisticated, flexible, and economical to maintain. Unlike the previous system, AGAP utilizes all the search and computational capabilities of both NCI computer systems (chemistry and biology computers) in a fully integrated mode.

The choice of groups for analysis is subject to one key constraint. Each group member must be definable and be accessible from the chemistry or biology computer systems. The choice then includes virtually any structural group and a host of non-structural elements involving suppliers, test systems, NSC ranges, etc. The choice is widened by use of Cri Codes. Any group can be made computer accessible by adding Cri Codes before NSC assignment. For instance, AGAP is used to analyze the results of the Hodes Model using Cri Codes. Every compound is coded according to the score it received from the Model; then each group with similar scorings are reported in terms of group performance.

AGAP calculates five separate group activity ratings for each category. A series of computing operations have been devised to characterize a group of compounds in terms of group size, current status in testing, activity observed between toxic group members as compared to non-toxic group members, relative performance in different test systems, different injection schedules, and different dose ranges; several factors reflecting the degree and reproducibility of activity, and initial sample size distribution within

each group. As with any numerical data, the significance and interpretation can vary with group size and other factors. Care is necessary to avoid over-interpretation and misinterpretation. In this respect, it is no different than any structure-activity study, statistical, biological, or physical measurement.

AGAP became operational in 1981. We have utilized this technique to analyze several substructures to enhance the effectiveness of our selective acquisition efforts. For example, a detailed analysis has been completed for quinones and tin complexes which will be presented in a forthcoming report.

B. Acquisitions Through Purchase Orders

A concerted effort was made during this report period to utilize the purchase order approach to acquire compounds of interest. The following chart provides the magnitude of this effort.

<u>Selected Compounds for Screening</u>	<u>Special Studies</u>	<u>Routine QNS Refills</u>	<u>Tumor Panel Candidates</u>	<u>Total</u>
878	13	21	55	967

All compounds acquired by the purchase-order mechanism for the screening program have been rated both by the Hodes Model and the Cri Codes. Nine compounds eventually qualified as candidates for broad spectrum tumor panel testing. Of these, NSC-322921, a chromosomal dye manufactured by Hoechst in Europe and distributed by Aldrich and by Calbiochem has a good potential of progressing through the Linear Array.

The perusing of latest catalogue offerings from custom synthesis houses has provided many new leads which are worthy of development. A few companies are willing to prepare analogues. However, they expect guaranteed financial support. The ideal solution for the development of these leads would be either through the Task Order mechanism or preferably through a resynthesis laboratory dedicated for the development of congeners.

The purchase order mechanism is providing some financial support to university scientists who are experiencing budgetary problems. The goodwill, thus created, will provide significant future benefits towards the voluntary submission of novel compounds for screening.

C. Automated Selection Through the Statistical-Heuristic Method

The computer-assisted preselection method is fully operational, i.e., computer-generated scores are now utilized to preselect the input of compounds to the P-388 prescreen. The independent results of two trials guided this decision. In addition, the integration of the Criteria Code System into the test-implementation stage evaluation process provided the close-monitoring capacity needed to assure timely evaluation of the test results.

The choices of computer-generated scores now implemented reflect the need to stress both structural novelty and the probability of activity against P-388. There are two novelty-related scores. The "common score" indicates that a compound is structurally similar to many compounds previously screened by

NCI. It is used for deselection. The "unique key" indicates that a compound has a structural fragment unique to the NCI file. This is used for positive selection.

The probability that a compound will be active against P-388 is scored numerically. Previous trials indicate that compounds which are scored very low are not often active against P-388. On this basis, compounds which score very low are deselected.

Recently, this method has been applied to predict the potential toxicity of new compounds for acquisition. Also, the applicability of this approach for literature surveillance has been explored utilizing the set of compounds registered at Chemical Abstracts Service during the course of a month.

II. Synthesis Activities (Tables 3 and 4)

DS&CB continued to pursue the DTP policy of deemphasizing regular contracts. The Task Order contracts became fully operational and provided several unique compounds for biological evaluation. The program responsibility for the two radiosensitizer contracts was transferred to the Radiotherapy Development Branch. However, the operational responsibility for these synthesis contracts remains with DS&CB.

Task Order Contracts - A New Initiative

The Quick Reaction Work Order contract mechanism represents an innovation in synthesis contracts. As such, a major effort was required this year to translate the basic concept into actual operating procedures including complex logistics, production, and administrative details. The Project Team approach was adapted to effectively manage this activity. This mechanism provides for the synthesis of a variety of organic and inorganic compounds which have been identified by Program as meriting development. Compounds selected for synthesis include panel compounds, presumptive actives, novel structures from literature sources, and compounds recommended by intramural scientists and approved by the Analog Development Committee.

The Task Order contract makes available ten master contractors (Table 3), who have the expertise to synthesize a wide variety of selected compounds. Each synthesis packet is sent to three master contractors and one contractor is selected for each task. The key element of technical excellence is optimized since three competing technical approaches can be evaluated for each packet. The incentive to continuously compete for each synthesis packet assures both technical excellence and cost-effectiveness of the Task Order mechanism.

For this reporting period, we have awarded 34 tasks consisting of 260 compounds for synthesis. Of these, the synthesis of 190 compounds (73%) were completed and the compounds were received during this period. Compounds selected for synthesis include PS actives, toxics, LE actives, special requests approved by the Analog Development Committee and panel compounds. Chart 1 shows the distribution of compounds selected for synthesis and the reasons for their selection.

To aid the Quick Reaction Task Order effort, it became necessary to establish a rapid mechanism for searching procedures for the synthesis of a wide

variety of compounds. To achieve this objective, the blanket purchase order mechanism was utilized and an agreement was worked out with Andrulis Research Corporation to provide on a regular basis the methods of preparation for compounds requested by DS&CB. This arrangement also provides for the chemical analysis of specific compounds selected for development. Since the identity, purity and physical properties of active compounds are critical, the data derived, which includes NMR, IR, UV, TLC, solubility, etc., have been extremely useful in establishing the correct structure and properties of compounds prior to the allocation of resources for further development.

Radiosensitizers

Radiosensitizers are chemicals that will selectively radiosensitize hypoxic tumor cells in combination with radiotherapy. The ideal substance would be expected to (a) mimic completely at non-toxic levels the radiosensitizing effect of molecular oxygen, (b) to diffuse rapidly into the hypoxic regions of tumors after administration, and (c) to be non-toxic to normal tissues. The objective of these contracts is to design, synthesize, evaluate and develop radiosensitizers with the goal of discovering compounds having a greater therapeutic efficacy (less neurotoxicity) as compared to Metronidazole and Misonidazole.

To date, SRI International has synthesized 136 target compounds. The physical-chemical parameters such as solubility, partition coefficients, reduction potential and electron affinity have been determined. In vitro and in vivo biological evaluations have been carried out on 40 of the compounds selected through structure-activity optimization studies. Two compounds, NSC-301467 and NSC-314055 have been identified by the Radiosensitizer/Radioprotector Working Group for further development. NSC-301467 is scheduled for preclinical toxicology. Recently, the group is focusing their attention on the synthesis and evaluation of potential non-nitro radiosensitizers. The N-oxide, NSC-25879, is found to have radiosensitization potency equivalent to Misonidazole with no detectable toxicity. The compound is undergoing an in-depth evaluation.

The Institute of Cancer Research contract has synthesized 137 1-and 5-substituted 2-nitroimidazoles and 2-substituted 5-nitroimidazoles. All were tested for in vitro cytotoxicity and radiosensitization enhancement ratios at different concentrations. Lipophilicity and electron affinity measurements were also determined. Selected compounds are undergoing in vivo evaluations. The hydroxylated nitroimidazole, NSC-328897, has been selected for further studies by the Radiosensitizer/Radioprotector Working Group since it did not exhibit neurotoxicity in the above test system. In addition, the contractor has actively supported our emerging program by measuring the electron reduction potential of 70 additional compounds identified by the Program.

The contractor has successfully developed a cytochemical technique in which changes in lysosomal enzyme levels can be measured directly in peripheral nerve preparations using scanning microdensitometry. This technique has been used to evaluate the neurotoxic properties of compounds that have demonstrated radiosensitization. The model has been adopted by its companion contractor, SRI International, to measure the neurotoxicity of promising compounds.

Natural Product Analogs

The objective of this contract is to develop, via chemical synthesis, compounds related to products of natural origin with improved anti-tumor activity and decreased toxicity.

The contractor has achieved a five step synthesis of bromo AT-125 using commercially available materials for the synthesis of bisimidates, then a sigmatropic rearrangement and finally, cyclization in the presence of a nitrile oxime. This method gives a mixture of the erythro and threo diastereoisomers which can be easily resolved. The bromo AT-125 will be used as an intermediate for the synthesis of peptides and dipeptides analogs. During the reporting period, the contractor has achieved the synthesis of the following; a blocked dipeptide of bromo homo AT-125, two glycine adducts, and a leucine analog. Some of these compounds have shown good biological activity but none of them is superior to the parent.

Another area is the synthesis of benzodiazepines related to anthramycin antibiotics. Early studies indicate that the pyrrolo ring of anthramycins might not be necessary for biological activity. Synthetic analogs include N-substituted and C-substituted benzodiazepines. Synthesis of several of these analogs has been achieved. To date, the N-propargyl benzodiazepanes appear to be the most active.

Nucleosides

The objectives of the contract are to design and synthesize novel nucleosides that inhibit key enzymes in both the de novo and salvage pathways of nucleoside metabolism, specifically HGPRTase, PNPase, and inosine dehydrogenase. The contractor has synthesized seven different amino and desamino sugars and four of them were converted to nucleosides. The biological evaluation of these nucleosides is in progress and two of them (NSC-340846 and NSC-339876) have been found to be inhibitors (K_i NSC-340846 = 1.2×10^{-4} M and K_i NSC-339876 = 0.5×10^{-3} M) of the enzyme PNPase.

III. Storage and Distribution

The objective of this project is the storage, distribution, inventory and documentation of synthetic materials, crystalline natural products and bulk clinical drugs. During the past year, Flow Laboratories, Inc., our storage and distribution contractor, has shipped more than 1,600 compounds per month to contract screening laboratories, formulation laboratories, NCI and NIH researchers, independent investigators in 44 states of the U.S. and 20 foreign countries. This represents a 23% increase in our distribution activity. The quantities of the shipped materials ranged in size from milligram to kilogram amounts. The vast majority of compounds are shipped within 48 hours after the receipt of the request and the packages normally arrive at their destination 48 to 72 hours later. Dry ice packages are transmitted only on Mondays and Tuesdays using the Government Bill of Lading or Federal Express. The bulk of the shipping is done using U.S. franked labels. The above outlined procedures have shortened the time between the shipping and the receipt of the compounds, and at the same time have reduced the shipping costs.

The contractor has begun gradually the physical inventory of the compounds (approximately 400,000 containers), held in the DS&CB repository in a priority order starting with Decision Network, Panel and SAC compounds. In February 1981, Flow implemented a computerized inventory system for Pharmaceutical Resources Branch bulk drugs to comply with FDA regulations for the accountability of Clinical Drugs. The contractor is also inventorying and systematically shelving compounds returned by the screening laboratories and reference samples. This activity is a continuing process and is limited by budgetary constraints.

The contractor interacts closely with the Drug Synthesis and Chemistry Branch, the Drug Evaluation Branch, the Natural Products Branch, the Pharmaceutical Resources Branch, as well as the acquisitions contractor and the chemical information contractor.

IV. Worldwide Surveillance

For years the NCI has acquired the majority of its compounds through the contributions of chemical and pharmaceutical industries, research organizations, and government laboratories. Both domestic and foreign sources have contributed generously in the past. Now, these contributions continue as a result of DS&CB's aggressive acquisition and worldwide surveillance programs, sustained good public relations and service.

The development of new sources is a continuing process necessitated by the dynamic nature of the chemical community. The diversity and availability of compounds for testing is heavily dependent upon existing research priorities in this community. The DS&CB must be able to anticipate and respond to such potential changes to ensure a continuous supply of unique compounds to the program.

During the past year, several new suppliers have been added to our program. The compound source analysis is depicted in charts 2 and 3.

We have developed a literature surveillance program, under contract, to expand and ensure the complete coverage of the chemical and biological literature to support our acquisition effort. The contractor previews the massive number of compounds published each month, estimated at 30,000 per month, and prepares a list of approximately 1,000 of the most interesting ones for review by DS&CB. These compounds are scored by the Hodes Model and reviewed by chemists. The compounds that are finally selected are acquired either through mail requests or Task Order synthesis.

Seminars

The following seminars were sponsored by DS&CB:

"Novel Pyrolizine Tumor Inhibitors, NSC-278214 and NSC-276702".
Dr. Wayne K. Anderson of the State University of New York, Buffalo.

"Thioxanthenones as Potential Anticancer Agents".
Dr. Sydney Archer of Rensselaer Polytechnic Institute, N.Y.

"Synthesis and Properties of Mesoinic Purinones; Potential Antineoplastic Agents".

Dr. Richard Glennon of the Medical College of Virginia

V. Chemical Searches

The search component of the Chemical Information System of the Drug Synthesis and Chemistry Branch plays an integral part in supporting the search needs of the various program elements of the branch, namely, the acquisition of new novel synthetics, contract synthesis and grants. In addition, the unit provides the chemical management data for other branches of DTP, the Data Review Subcommittee, the Drug Evaluation Committee, and the various analog committees. It also provides the scientific community with chemical information about the chemotherapeutic agents in the Program.

The substructure search feature of the Chemical Information System provides data necessary to determine Program relevance, the exploration of new areas for development and the identification of areas for deemphasis. The data are also useful in the development of new research projects and structure-activity studies.

During this reporting period more than 250 substructure searches were performed. Twenty four percent of these were in response to requests from the Data Review Subcommittee or Prescreen Committee, 17% from the Drug Evaluation Committee, 14% from extramural scientists, 16% from miscellaneous sources and 18% in support of the Branch's activities. Chart 4 shows the number of searches and sources of these requests.

The full structure search capability of the Chemical Information System (CIS) determines the uniqueness of specific compounds, and the detection of duplicates in support of the Branch's selective acquisition program, other branches of DTP, and extramural and intramural researchers. For the reporting period, full structure searches totaling more than 1,200 were performed.

VI. Status of QNS Panel Compounds

DS&CB, with the cooperation of DEB and the support of our contractors, initiated a major effort to streamline the backlog of QNS compounds, reported to be around the range of 1,900 compounds. Through a concerted year-long effort, we have reduced the level of QNS panel compounds to 409, 317 being synthetic compounds. Thus, we have reached a dynamic equilibrium state.

The profile of the panel QNS to date is summarized on Table 5.

TABLE 1

CANCER RESEARCH EMPHASIS GRANTS

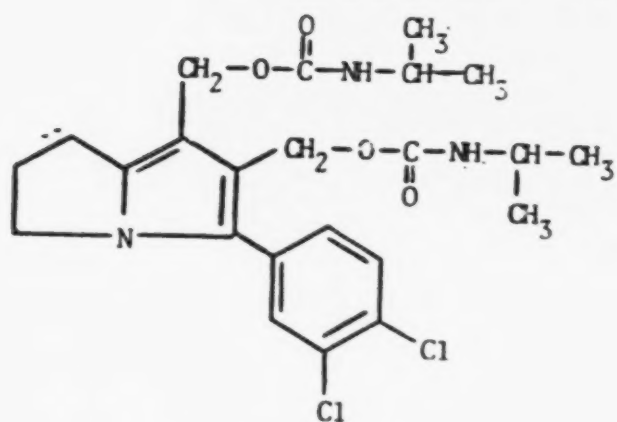
<u>Institution</u>	<u>Investigator</u>	<u>Grant Number</u>
Brandeis University	Abeles	RO1 CA 23496-03
Louisville, Univ.of	Huang	RO1 CA 25252-03
Sidney Farber Cancer Institute	Rosowsky	RO1 CA 23151-03
Southern California, University of	Heidelberger	RO1 CA 25715-03
Southern Research Institute	Shealy	RO1 CA 23127-03
Southern Research Institute	Temple	RO1 CA 23141-03
SRI International	Acton	RO1 CA 25711-03
Yale University	Canellakis	RO1 CA 23153-03

T A B L E 2

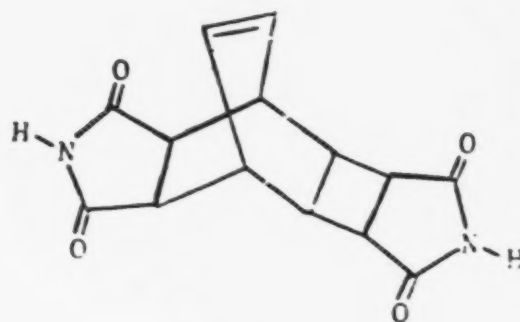
SYNTHETIC COMPOUNDS ASSIGNED TO DN₂

<u>NSC Number</u>	<u>Name</u>
40774	6-Methylmercaptapurine riboside
146068	Platinum, (ethylenediamine) (malonato)
233853	Adriamycin, octanoylhydrazone
241240	Platinum, diamine[1,1-cyclobutanedicarboxylato](2-)- <u>0</u> , <u>0</u> ¹ -
253272D	
256927	Platinum, dichlorodihydroxy bis(2-propanediamine)-(OC-6-33)-
266210	Bisdaunorubicinhydrazone
267702	Cyclohexyl pyrrolizine derivative
268242	N-Dibenzyl-daunorubicin
271674	Platinate(1-), [1,2,4-benzenetricarboxylato(<u>3</u> -)- <u>0</u> ¹ , <u>0</u> ²](1,2-cyclohexanediamine-N,N')-hydrogen
278214	Isopropylpyrrolizine derivative
284356	4,8-Ethenopyrrolo[3',4':3,4]cyclobut[1,2-f]isoindole-1,3,5,7(2H,6H)tetrone, octahydro
286193D	
305884D	
327471D	
337766	Orange Crush
404241	Ara-A

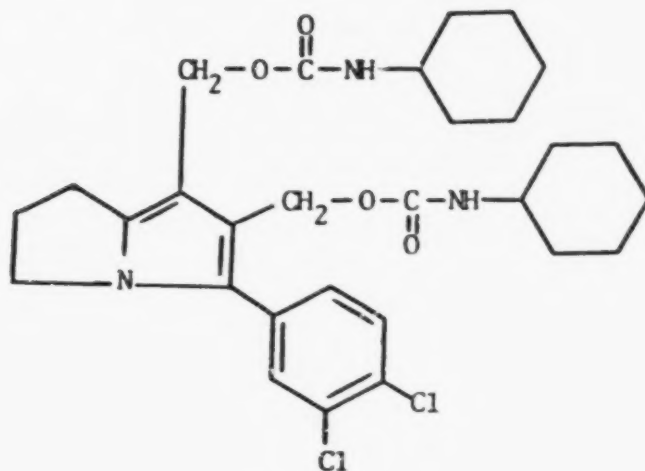
C H E M I C A L S T R U C T U R E S



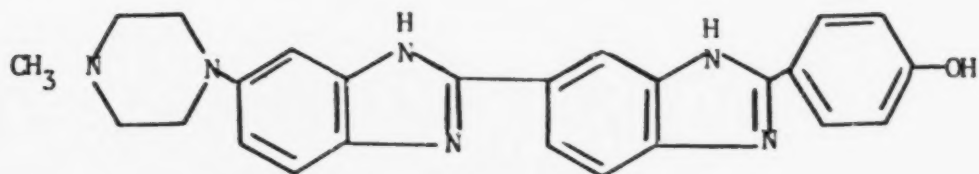
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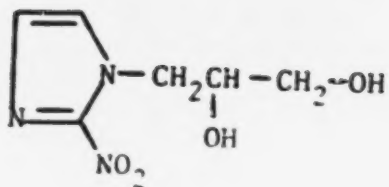
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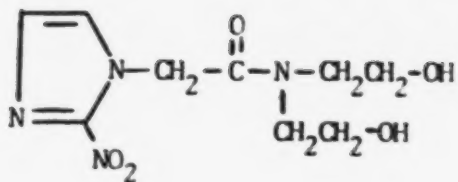
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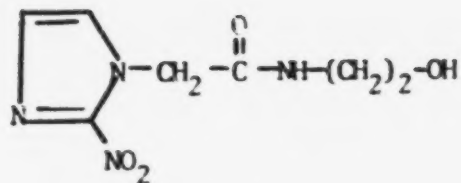
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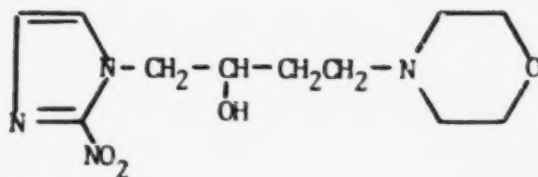
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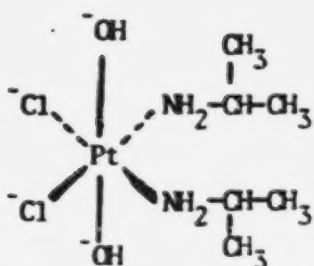
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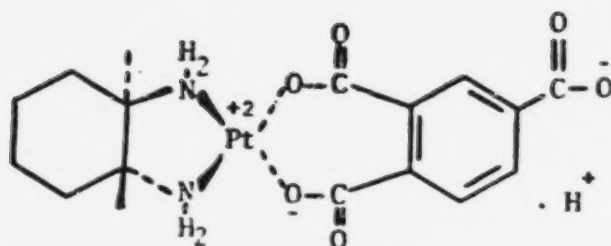
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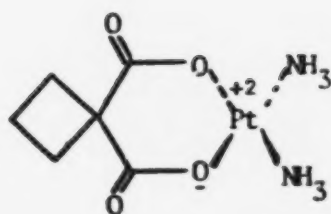
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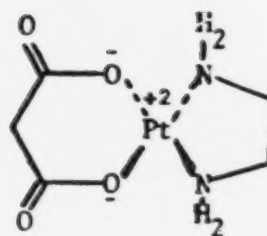
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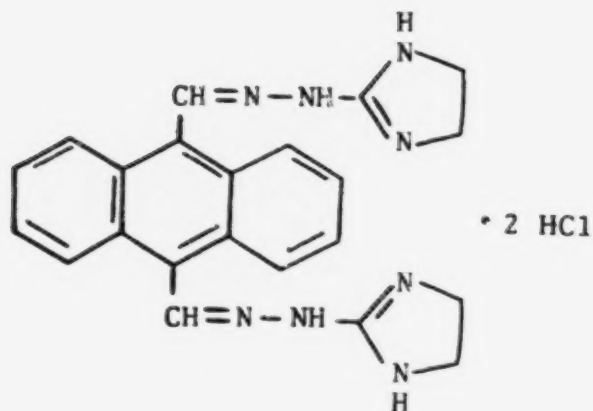
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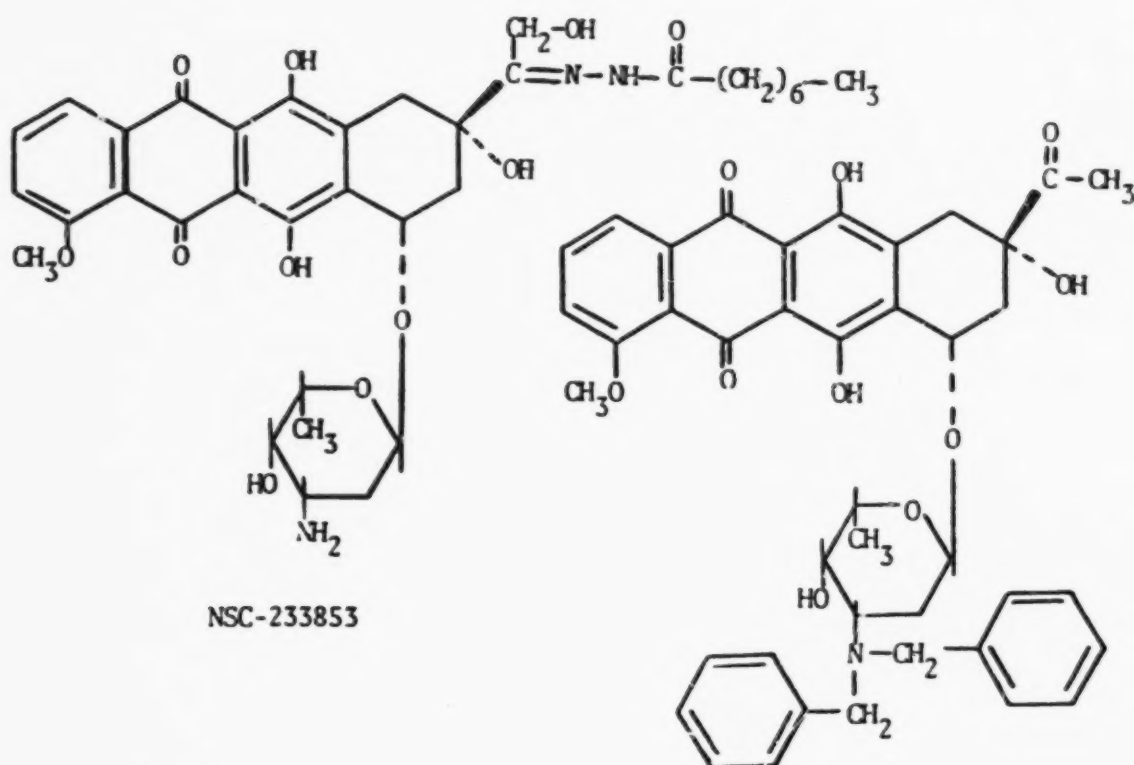
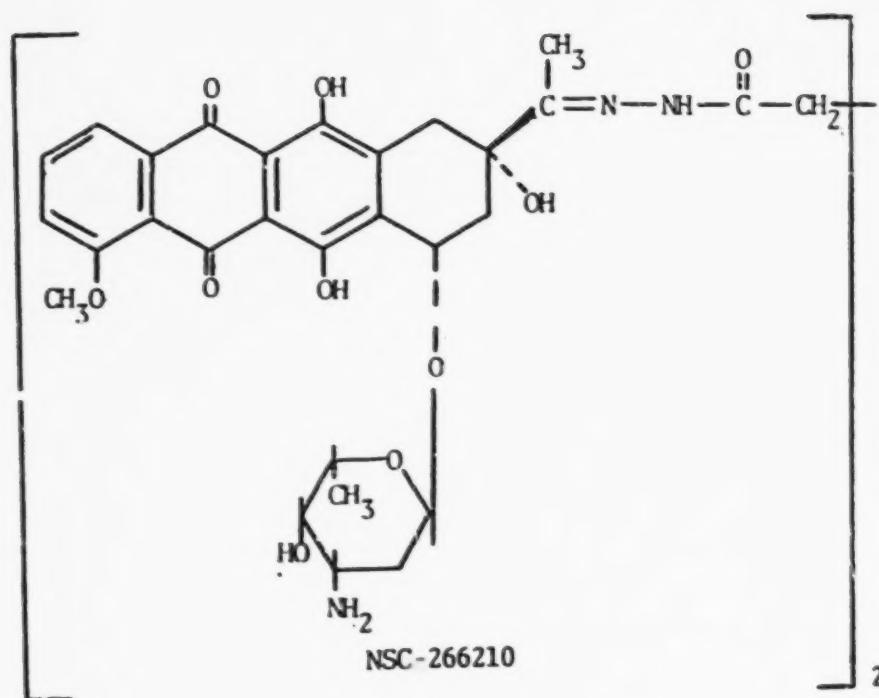
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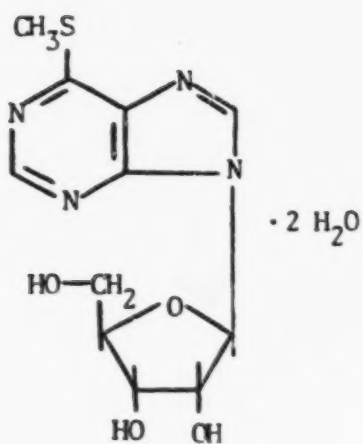


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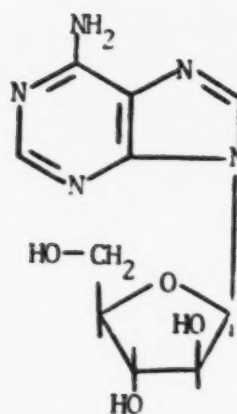


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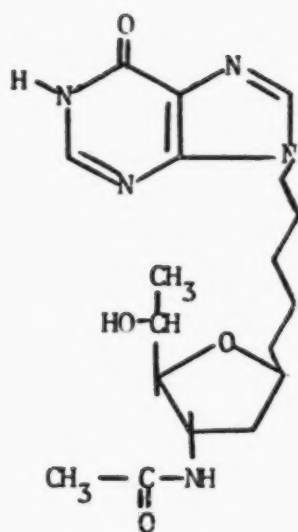




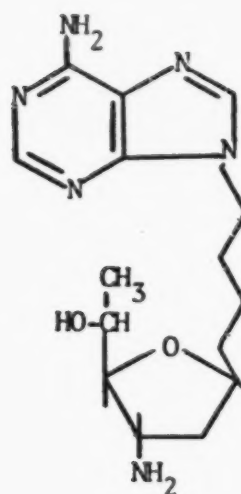
NSC-40774



NSC-404241



NSC-340846



NSC-339876

TABLE 3
QUICK REACTION WORK ORDER CONTRACTS

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Alabama, University of	Baker	N01-CM-07355
Collaborative Research, Inc.	Friedman	N01-CM-07358
IIT Research Institute	Ucich	N01-CM-07359
Mid-America Cancer Center	Cheng	N01-CM-07353
Research Triangle Institute	Seltzman	N01-CM-07352
SISA, Inc.	Razdan	N01-CM-07354
Southern Research Institute	Montgomery	N01-CM-07260
Southwest Foundation for Research and Education	Rao	N01-CM-07356
SRI International	Acton	N01-CM-07351
Starks Associates, Inc.	Starks	N01-CM-07357

TABLE 4

DRUG SYNTHESIS & CHEMISTRY BRANCH CONTRACTS

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Bristol Laboratories	Doyle	N01-CM-87180
Chemical Abstracts Service	Myers	N01-CM-43722
Flow Laboratories, Inc.	Dorian	N01-CM-97254
Institute of Cancer Research	Adams	N01-CM-77139
Michigan Technological University	El Khadem	N01-CM-07293
SRI International	Lee	N01-CM-87207
Starks C. P., Inc.	Schultz	N01-CM-87206

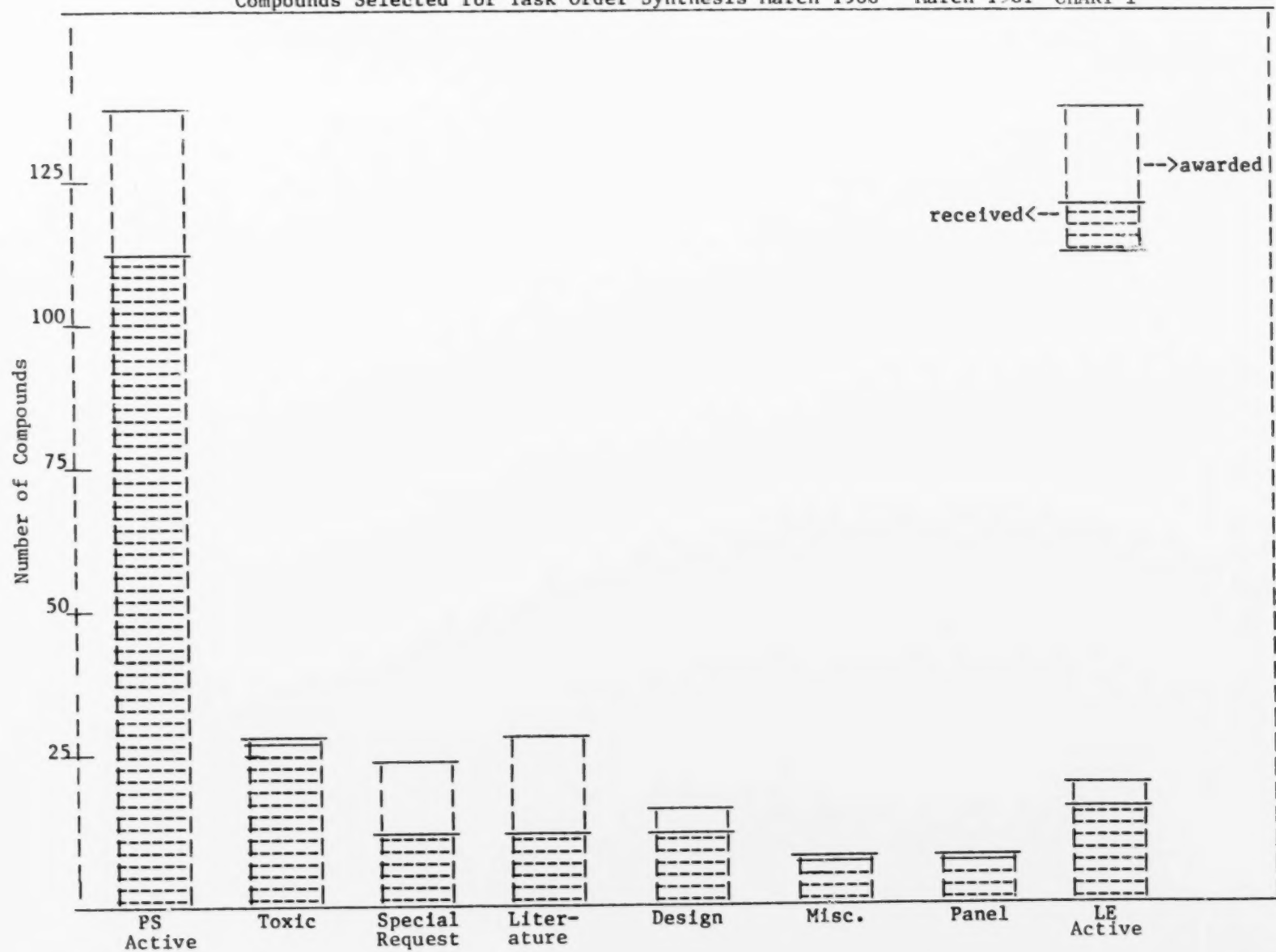
TABLE 5

Status of QNS Panel Compounds

Assigned to Prep. Laboratories.....	41
Assigned to Task Order Contracts.....	7
Initiated Purchase Orders.....	13
Under active solicitation from:	
(a) Industrial Suppliers.....	164
(b) Research Institutes, Individual Suppliers, etc.....	<u>92</u>
Total	317

BEST DOCUMENT AVAILABLE

Compounds Selected for Task Order Synthesis March 1980 - March 1981 CHART 1

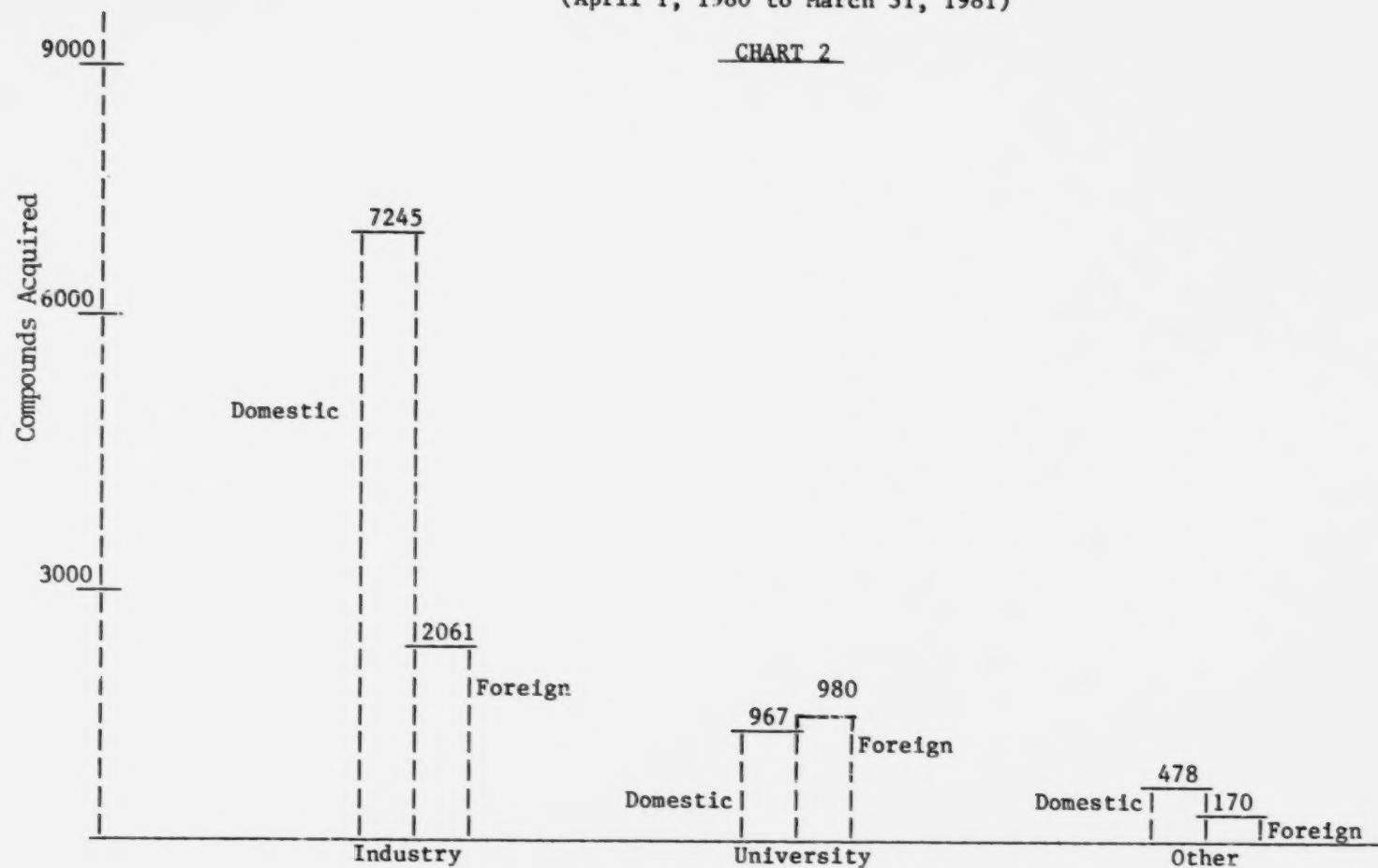


223

COMPOUND SOURCE ANALYSIS - FOREIGN & DOMESTIC

(April 1, 1980 to March 31, 1981)

CHART 2



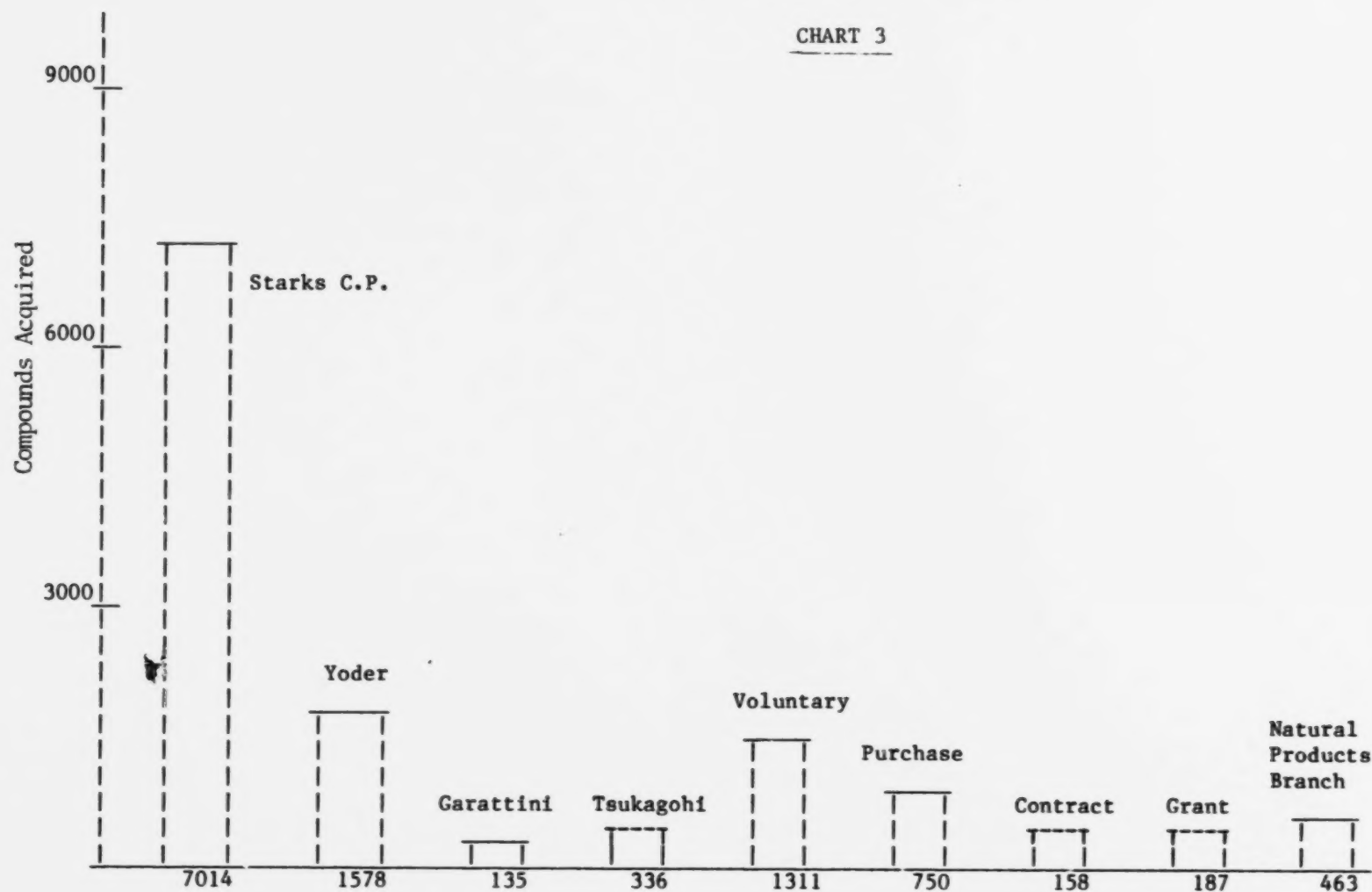
BEST DOCUMENT AVAILABLE

223

COMPOUND SOURCE ANALYSIS

April 1, 1980 to March 31, 1981)

CHART 3

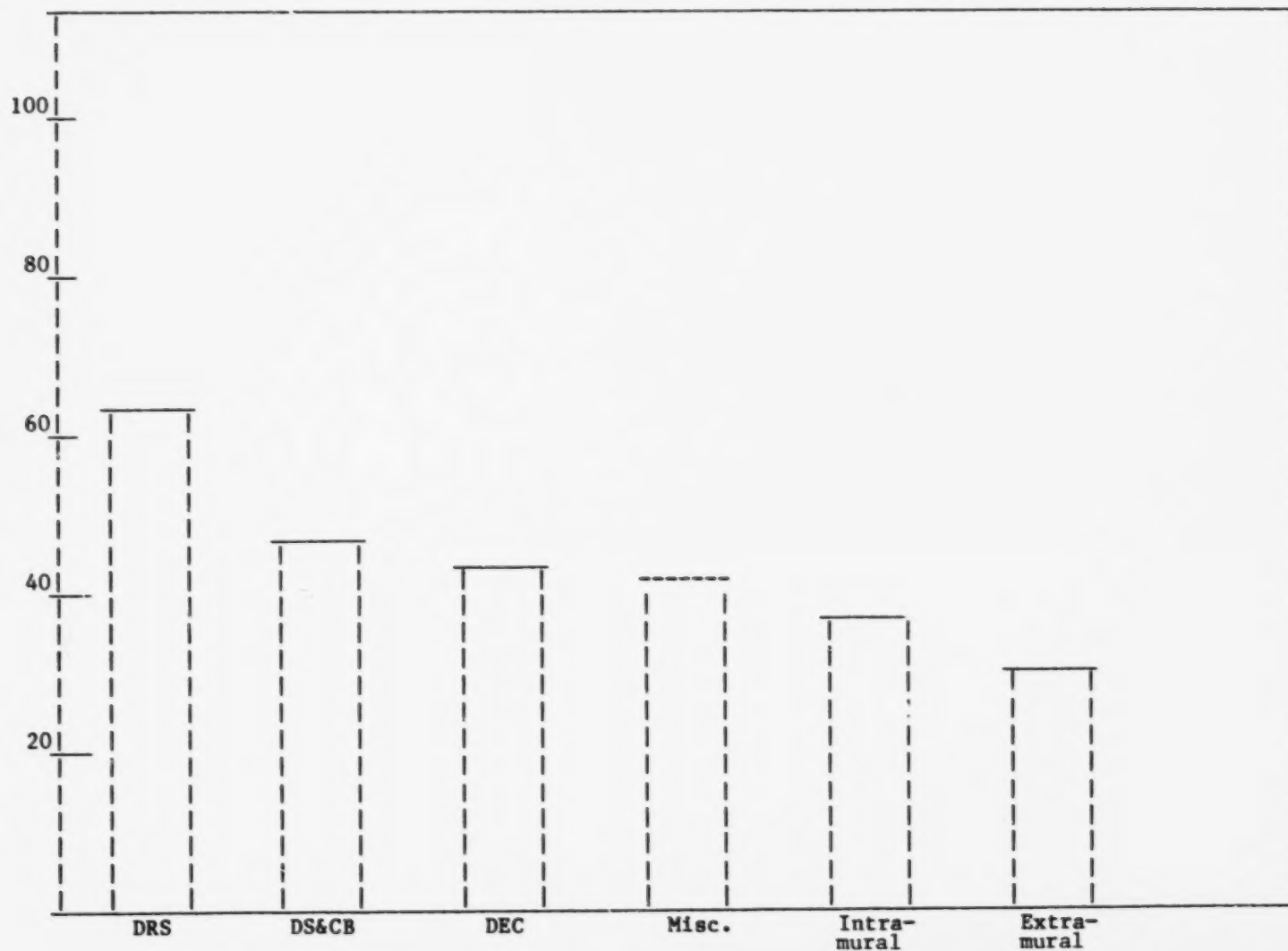


BEST DOCUMENT AVAILABLE

284

SUBSTRUCTURE SEARCHES

MARCH 1980 - MARCH 1981 CHART 4



225 BEST DOCUMENT AVAILABLE

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM-07101-06 DSCB																
PERIOD COVERED October 1, 1980 to September 30, 1981																		
TITLE OF PROJECT (80 characters or less) Computer Methods for Drug Preselection Based on Structure-Activity																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 10%;">PI:</td> <td style="width: 20%;">L. Hodes</td> <td style="width: 40%;">Research Mathematician</td> <td style="width: 30%;">DS&CB, NCI</td> </tr> <tr> <td>Other:</td> <td>K. Paull</td> <td>Chemist</td> <td>DS&CB, NCI</td> </tr> <tr> <td></td> <td>P. Blower</td> <td>Chemist</td> <td>CAS</td> </tr> <tr> <td></td> <td>R. Geran</td> <td>Biologist</td> <td>DEB, NCI</td> </tr> </table>			PI:	L. Hodes	Research Mathematician	DS&CB, NCI	Other:	K. Paull	Chemist	DS&CB, NCI		P. Blower	Chemist	CAS		R. Geran	Biologist	DEB, NCI
PI:	L. Hodes	Research Mathematician	DS&CB, NCI															
Other:	K. Paull	Chemist	DS&CB, NCI															
	P. Blower	Chemist	CAS															
	R. Geran	Biologist	DEB, NCI															
COOPERATING UNITS (if any) Chemical Abstracts Service																		
LAB/BRANCH Drug Synthesis and Chemistry Branch																		
SECTION Chemical and Drug Information Section																		
INSTITUTE AND LOCATION NCI, NIH, Silver Spring, Maryland 20910																		
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">TOTAL MANYEARS: 1.0</td> <td style="width: 33%;">PROFESSIONAL: 1.0</td> <td style="width: 33%;">OTHER: 0</td> </tr> </table>			TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0													
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0																
CHECK APPROPRIATE BOX(ES) <table style="width: 100%; border: none;"> <tr> <td>☐ (a) HUMAN SUBJECTS</td> <td>☐ (b) HUMAN TISSUES</td> <td>☒ (c) NEITHER</td> </tr> <tr> <td colspan="3">☐ (a1) MINORS ☐ (a2) INTERVIEWS</td> </tr> </table>			☐ (a) HUMAN SUBJECTS	☐ (b) HUMAN TISSUES	☒ (c) NEITHER	☐ (a1) MINORS ☐ (a2) INTERVIEWS												
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☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) A <u>quantitative structure-activity correlation</u> method has been designed to cover <u>large amounts of data over diverse classes of compounds</u>. The method is used to aid in <u>selecting compounds for screening in mouse lymphocytic leukemia (P388)</u>. Estimates of activity, novelty, and more recently, <u>toxicity</u> are based on P388 test results from over 50,000 compounds. The <u>activity estimate</u> is derived from the <u>statistical significance</u> of the incidence of each structural characteristic in the active compounds. The <u>novelty estimate</u> is based on the incidence of structural characteristics in previously tested compounds. These estimates are examined by the medicinal chemist along with the structures for about 30,000 potential acquisitions per year to decide which compounds to acquire. Active use of this process began in April 1980 after some experimentation to validate the method. Also a pilot run of a <u>literature surveillance</u> version was applied to one month's <u>CAS registrations</u>.																		

Objectives:

1. Evaluate the potential of structure-activity determination over broad ranges of compounds for specific biological test systems.
2. Demonstrate feasibility of such methods to preselect compounds for anti-tumor screening, e.g., in setting priorities or in selecting appropriate test systems for screening.
3. Within the screening capacity, maximize the acquisition of additional promising compounds as well as compounds inadequately represented in the file.
4. Install these methods into the Chemical Information System (CIS) operating environment.
5. Adapt these methods for literature surveillance by processing all CAS registrations.

Methods Employed

1. The method for predicting activity uses the incidence of each molecular fragment feature in compounds of known activity to compute an activity weight for the feature. The collection of compounds of known activity is called a training set. Upon completion of the training phase, i.e., the computation of activity weights, these weights can be applied to new compounds according to the fragments they contain. Reference (1) describes the method and compares its performance to other prediction methods. In reference (2) the method is applied to the large volume of P388 data as a means of finding new active classes. Reference (3) reports work done last year to help validate the method.
2. Biological data, thus far limited to the P388 test system, is obtained by means of a query of the DTP Biology File. Active compounds are collected in two categories according to T/C, the ratio of the median life span of the treated mice to that of the controls. The A category requires a confirmed T/C of at least 175% and the C category requires a confirmed T/C of at least 120% but not category A. The inactive compounds (N's) require a regimen of at least two injections and at least three dose levels, the lower two non-toxic. The update installed September 1980, contained 1,225 A's 3,963 C's and 48,232 N's.
3. Molecular structure fragments including ring and nuclei keys and smaller fragments known as ganglia augmented atom keys, are being employed as predictor variables. Reference (4) discusses the selection of these fragments, which were originally designed for substructure search of the DTP Chemical Information System. There are about 30,000 distinct fragment features in the P388 data. The model for activity uses only the 6,500 features occurring in the active compounds.

4. A table containing the incidence of the roughly 30,000 fragment features in P388 testing is employed in order to obtain a measure of novelty of a new compound. When a potential acquisition is processed, its feature of least incidence in P388 testing is found along with the incidence. If the compound has features new to P388 testing, these are reported as unique.
5. A search of the DTP Biological File was performed to provide data for a P388 toxicity model. 3,653 compounds were found which were toxic at 50 mg/kg and all higher doses. Corresponding to the inactive training set were 29,841 compounds, non-toxic at 200 mg/kg and all lower doses.
6. Also in the preselection module, but not developed under this intramural research project, are a series of about 100 profiles of chemical classes. These are defined by special structure features (SSF). Most of the SSF were selected from the 200 or so recognized SAC classes and about one-fourth define classes DTP is following as CRIT codes.
7. The above methods led to an operational preselection module first installed in April 1979. The activity score and novelty measure, although not used for selection during the first year, were recorded as default CRIT codes in those acquisitions which did not fit other CRIT codes.

Based on early results from this testing and the independent experiment involving 1000 unselected compounds reported last year (reference 3) the model began employment in April 1980 in the following manner.

- A. All compounds having a unique feature are almost automatically accepted.
 - B. All compounds which have each feature occurring at least 50 times in P388 testing are considered candidates for elimination as adequately represented.
 - C. All compounds which have an activity score in the lowest tenth of the training set range are considered candidates for elimination as probably inactive.
8. An experiment in literature surveillance was performed under which one month's CAS registrations, about 30,000 compounds, were run to determine the following:
 - A. An estimate of activity was determined in a version that used a newly developed set of features (see last year's annual report).
 - B. An estimate of novelty was defined as only those compounds with unique features.
 - C. SSF which were defined in paragraph 6 above.

Structures are obtained for those compounds satisfying combinations of criteria A, B, and C. From these structures compounds are selected to attempt acquisition.

1. From April 1979 to April 1980 CRIT codes were used to track activity and novelty scores, each in three intervals. Low activity comprised scores below the third decile, moderate activity between the third and sixth, and high activity above the sixth decile according to the scores in the training set. The great majority of the compounds fell in the low activity code mainly because the compounds acquired were poorer than usual but also because the lowest deciles are normally populated in a much higher proportion than in the training set. (See reference 3). The novelty measure was also divided into three categories according to the incidence of the least occurring feature. Low incidence or high novelty, 0 to 3 occurrences, medium, 4 to 30 occurrences, and high incidence or low novelty over 30. Here there was more success, with the results showing that the low novelty compounds had a yield of confirmed actives about half that of the other two categories. See Table I. This reinforces the finding in reference 3 that compounds showing as adequately studied have remarkably low activity.

Results on compounds selected since April 1980 are recorded according to decile by ten CRIT codes which were in effect until September 24, 1980 when the model was updated. The biological data is shown in Table II. Note that the number in each decile range is more comparable since the lower ranges were deselected. The results are not yet very conclusive; indeed are not able to show much because deselected compounds were not tested. However, the total yield of actives is likely to improve when more data are received. Biological data since the September update are not sufficient to compare.

2. The pilot run of the surveillance model produced more compounds than anticipated. Of the 30,000 CAS registrations 7,000 had features unique to P388 testing and 7,900 met one or more of the SSF criteria, with one SSF yielding over 2,000 compounds. Because of some overlap, the number of compounds containing an SSF and/or a unique feature was 13,200. It had been anticipated that roughly 6,000 compounds would require structures to be reviewed, including those with high activity score, in this pilot run.

Therefore it was decided to review only those structures which satisfied at least two of the criteria:

- A. One or more unique features.
- B. One or more SSF after eliminating all but 500 from the SSF containing over 2,000 compounds.
- C. In the top half of the activity score, i.e., in the top 15,000 compounds.

The first batch chosen were SSF's with unique features. (See table III). 1,716 structures were reviewed in this category, including those meeting all three criteria. It was observed that most of the unique features belonged to metallic compounds of little interest. Indeed only 76 structures were selected to be pursued further. Of the remaining 5,300 compounds with unique features but no SSF, 3,781 were in the top 50% of activity score including about 900 with 0 activity score due to a lack of any features from the training set. These 3,781 were further subdivided at DTP request according to whether the unique features contained metal atoms. There were 1,528 compounds with all their unique features having metal atoms, 1,892 compounds which had only non-metal features and 361 compounds which had both. The 1,892 compounds were chosen as the next batch. Of the remaining 4,700 SSF compounds with no unique features, 1,732 were in the top half according to activity score. These were to be the third batch to review.

Unfortunately, this approach to literature surveillance has received a setback due to lack of resources and the failure of CAS to win the competition for a contract to perform literature surveillance. Abstracts were obtained only for 59 of the first batch of 76 structures and these are being followed through.

3. The toxicity model was created from 80% of the toxicity data described in paragraph 5 of the methods section. The remaining 20% of each group (718 toxic and 5,878 non-toxic compounds) were run through the model as a test set. Table IV shows the number of "unknown" toxic compounds receiving scores in each tenth when the test set is ranked by tenths. Note that 39% of the toxic compounds fell into the highest tenth, where the ratio of non-toxic to toxic is lowered from 8.2/1 overall to 1.3/1. It is expected soon to begin testing at lower dose levels compounds rated highly toxic.

Proposed Course

1. The utility of these methods as an aid in selection will continue to be monitored.
2. If the experiments on literature surveillance have a favorable outcome, all CAS registrations, roughly 400,000 compounds a year, can be processed. This is limited by resources.
3. The feasibility is being checked of some other types of features based on physical constants such as partition coefficients. If such constants can be computed on the required large scale they can be useful in conjunction with other, structural, features.
4. When a sufficient number of structurally varied compounds which are active in the P388 prescreen have been tested in the Division of Cancer Treatment (DCT) panel of screens, models will be developed and tested to optimize the testing of such compounds, e.g., when the supply of materials or the screening capacity is limited.

Significance for Biomedical Research and the Program of the Institute

1. The enrichment effect produced by preselecting compounds should increase the number of actives within the current screening level and maximize the potential of screening compounds with unique structural characteristics not adequately represented in the National Cancer Institute (NCI) collection.
2. The method developed for the prediction of probability of activity is now recognized as a contribution to the state-of-the-art of broad range structure-activity. It can be applied to many areas, e.g., using carcinogenicity or toxicity as the biological activity. Copies of the program and documentation have been requested by outside investigators, at least 2 of whom have been using the method. CAS is planning to incorporate these methods as a service to customers.

Publication

- (1) Holes, L: Computer-Aided Selection of Compounds for Antitumor Screening. Validation of a Statistical-Heuristic Method. J. Chem. Inf. Comput. Sci., in press.

T A B L E I

Biological Results* From Compounds Rated From April 1979 To April 1980

A C T I V I T Y

		LOW	MEDIUM	HIGH	TOTAL
N	HIGH	8/1045	2/134	4/156	14/1335
O					
V	MEDIUM	8/1015	0/91	0/89	8/1195
E					
L	LOW	5/793	0/22	0/40	5/855
T	TOTAL	21/2863	2/247	4/285	27/3385
Y					

*Results are given as ratio of confirmed/confirmed + failed for all compounds which finished P388 screening.

Z01 CM-07101-06 DSCB

T A B L E I I

Biological Results From Compounds Rated From March 1980 To September 1980

TENTH	CONFIRMED	CONFIRMED + FAILED
1	0	55
2	1	271
3	0	204
4	2	178
5	0	197
6	0	261
7	1	258
8	1	196
9	3	151
10	4	99
TOTAL	12	1,880

T A B L E I I I

Yield Of Structures From Pilot Run Of Literature Surveillance Model On 30,000 Compounds

	SSF	NOVEL
	7,900	7,000
BOTH	<u>1,716</u>	
REMAINING	4,700	5,300
TOP 50% ACTIVITY	<u>1,732</u>	3,781
NON-METAL		<u>1,892</u>

Z01 CM-07101-06 DSCB

T A B L E I V

First Toxicity Model Run

Tenth	<u>Toxic</u>		Non-Toxic
	Number	Cum. %	
10	283	39	377
9	107	54	553
8	81	66	579
7	62	74	598
6	48	81	612
5	36	86	624
4	37	91	623
3	28	95	632
2	16	97	644
1	20	100	636
TOTAL	718		5,878

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REPORT OF THE CHEMICAL & DRUG INFORMATION SECTION
DRUG SYNTHESIS & CHEMISTRY BRANCH (DS&CB)
DEVELOPMENTAL THERAPEUTICS PROGRAM (DTP)
DIVISION OF CANCER TREATMENT (DCT)

I. SCOPE

The Chemical & Drug Information Section (C&DIS) of DS&CB has the responsibility of providing DTP with the capability to manage its chemical information.

For this purpose, C&DIS maintains a computerized chemical information system. The system monitors accession, storage and distribution of chemicals acquired under the program. It maintains inventory control, and tracks acquisitions from receipt through shipment to the screening laboratories.

It identifies duplicates, detects various errors, and selects, from its input, analogs of Program interest. It communicates this information to DTP personnel, to contractors and to other systems, in particular, to the Biology data processing system.

The system offers online access, and allows query definition on the basis of full structure, substructure, and other data elements.

A nomenclature index, referencing compounds in the system, is periodically generated. It includes comprehensive listings of synonyms.

Finally, the system lends itself to data analysis and modelling. Work in this area has included the prediction of antitumor and toxic activities, the determination of the novelty of compounds, and the prediction of the yield of actives to be obtained from classes of predefined compounds. The usage of the system thus ranges from day to day logging or shipping activities, to the chemical, physical, biological and statistical evaluations on which depend the selection of antitumor compounds for, and their progression through, the Linear Array.

The system is operated under contract N01-CM-43722 by the Chemical Abstracts Service (CAS) in Columbus, Ohio. To date, it has accumulated data on over 360,000 chemical compounds, and recognizes, independently of chemical structural characteristics, more than one hundred different data elements.

The Section is composed of one systems analyst, a technical information specialist, a chemical data clerk, and a part-time stay-in-school clerk.

II. HIGHLIGHTS OF FY 1981:

In the past year, the system was greatly developed. Users from another branch (LMCB), with their own terminal, were allowed to tap into the system. Various improvements, such as the inclusion of Chemical Abstract's

Registry Numbers and the speeding up of NSC number assignment, were implemented. But this section's major effort was devoted to the establishment of an interlink between the chemical and biological data on file. As a result, biology reports now can be provided with the chemical structure to which the data pertain. And users of the online chemical system can obtain, associated with the structures retrieved, a summary of their biological data.

On the negative side, the CAS contract, under which the chemical system is operated, increased in cost from about \$450,000, a level at which it had remained for the past several years [excepting \$80,000 added last year for the interlink], to \$826,355 for the current contract year (assuming the same resources), with further increases for subsequent years.

A. The Interlink.

As mentioned, the major effort of this section, this year, was devoted to overcoming the functional separation of the chemical and biological systems. This had been a cause for concern for many years. The Jacobus Committee, convened in 1979 to determine whether the DTP information management system was within the current state of the art, felt that it was not, that it placed, instead, serious limitations on the productivity of NCI program managers who were forced to adapt to it. Prominent among the system's deficiencies was the functional separation of the two files.

The poor linkage between the NCI's chemistry and biology systems reflected profound differences existing between these two. These differences are partly inherent in the nature of the data, partly the result of the separate organizational management of the two systems. The chemical system is run on a computer in Columbus, Ohio; the biological system on a machine located at DCRT, in Bethesda. By policy, no host-to-host communication can be established between these two machines. The chemical system further operates in interactive mode, providing responses immediately upon inquiry. The biology system operates in batch mode, requiring a turn-around time of several days to answer a query. The chemical structure output is coded in the form of line vectors. The biology output is represented by standard characters. Few printers are manufactured that can handle both outputs, and those that do are slower and may require special (chemically treated) paper. Because of these differences, the outputs from the Chemistry and Biology systems have always been obtained separately.

The short-range solution, developed by this section to resolve the Interlink problem, consisted of two approaches:

- A "basic" Interlink, allowing the recipient of any Biology report (generated by DEB) to obtain it with a chemical structure inserted against each NSC number listed.

- An "interactive" Interlink, providing a user of the interactive Chemistry file (who will compare many structures) with appropriate biological data to evaluate the activity of retrieved compounds.

To implement the "basic" Interlink, a copy of all structures on the CAS file (more than 355,000) was transferred to a disk file at DCRT, which is updated bi-weekly. Any biology report, henceforth processed at DCRT, can fetch the corresponding structures from this file.

To print the reports on which graphic data (chemical structures) and text (biology data) are intermixed, a printer/plotter (VERSATEC 1200) was procured, together with an offline Raster Processor (VERSATEC 312), required to drive this device. Because the VERSATEC equipment is designed to print either graphics or text, but not both intermixed, some modification of vendor hardware and software was required. In addition, programs to convert chemical structures, from CAS file-format to VERSAPLOT input-format, had to be installed on the DCRT computer. Fig. 1 represents an example of the output produced with the completed setup.

To obtain biology data corresponding to search results from the chemical system, the latter was modified to collect, upon request by the user, the outputs of GET, SSS and FSS searches. These are collected on a "B-tape" which is forwarded, bi-weekly, to the Biology System, where the desired reports are then produced.

Online searches having requirements different from the above, the approach to the "interactive" interlink was different as well. Online information is quickly viewed, just long enough to determine what needs to be requested next. Such information thus is normally brief. Exhaustive listings can always be obtained offline, on paper. Further, random access memory is limited. For these reasons, it was advisable not to bring all the available Biology data to the interactive interlink, but only a summarized subset thereof.

The data elements comprising the subset were selected, in a number of deliberations, by the Interlink Committee. In accordance with the consensus reached, the data for each treatment schedule were summarized on one line displayable by computer terminal. Each line includes test system, treatment schedule, route of administration, etc. For viewing, the lines pertaining to each NSC number appear sorted in that order. A sample of this output is shown in Fig. 2.

Approximately 600,000 records, summarizing the entire biology file, and each representing a display line, have been transferred to the online chemical file, where they will be periodically updated.

At present, these data are available only for display. To make them searchable is a task to be completed in the forthcoming year. It will then be possible to put queries to the system such as the following:

- What is the experience, to-date, in testing amides of thiadiazoles against 3PS31?

- Which cephalosporins have exhibited a significant T/C (previously defined as at least 150) when tested in the 3B131 system?
- Which Adriamycin derivatives are available to send 2 grams for testing that have a significant T/C (at least 190) in 3PS31 or a significant T/C (at least 150) in 3B131?
- Retrieve all compounds with a given substructure that were never tested in a particular system
- Retrieve all compounds that were active in a given test system, but not in another.

B. Data analysis and modeling of antitumor activity.

The existence of a concentration of chemical and biological data in the area of antitumor activity presents a unique opportunity for data analysis. To take advantage of this, a computerized model (Hodes) has been perfected over the past several years, to determine:

- the likelihood that a compound will possess antitumor activity.
- the likelihood that a compound will possess toxic activity.
- the presence of structural characteristics not found in other compounds in the program, thus providing a measure of rarity or uniqueness.

In the past year, the above model has been installed as part of the operational system, where it is routinely used for the evaluation of potential acquisitions.

Extensive "ground truth" tests of the validity of the model were performed, and have been published.

Independently, a method (AGAP) was implemented to correct for the bias that obtains in recently tested compounds, where negative results, being much sooner available than confirmed actives, predominate. The method projects the yield of confirmed actives for given groups, taking the anticipated negatives into account.

At present, the method is implemented offline. The interactive interlink, however, will allow for the computation, on the fly, of a significance indicator for the group defined by current search criteria.

Details of these methods are reported on separately.

III. FUTURE PLANS.

The imminent formation of the Information Technology Branch, from components of DEB and DS&CB, will bring under one roof the administration of the chemistry and biology files. That will focus attention on the desirability of redesigning the chemical and biological systems, to form an up-to-date, homogeneous and efficient system.

As mentioned, the biology system is operated in batch mode, independently of the chemical system. Conversion of the Biology data to online access would be of considerable benefit to the user community, would greatly reduce duplication (e.g. it would make unnecessary the data summaries on the interactive interlink file, the offline SAC file, etc.), and would simplify coordination with the chemistry data.

If a redesign were to be undertaken, it would be justified not solely on behalf of the biology system. The chemistry file, too, is greatly in need of rehabilitation. The system is almost 10 years old. In these years, inevitably, the original plans were considerably modified, partly by the staged addition of planned capabilities, partly by the installation of initially unanticipated enhancements, such as the SSF's the non-structural keys, the search qualifications and, last but not least, by the installation of an interim interlink. These have rendered the system cumbersome and expensive to operate.

For example, delays in excess of a month have been experienced in registering a group of compounds. This is a result of the batch updating of the files. Online updating is difficult with inverted lists, which is the file organization adopted by our system. The large number of keys used by a chemical system preclude an alternative file organization. The difficulty might be mitigated through the use of a commercially available data base management system, such as ADABAS or Model 204, where these problems have already been solved. Such systems will therefore be investigated. Possibly, they could accomodate both the chemistry and the biology systems.

It is the large volume of biology data which, thus far, has precluded them from being placed on disks. A suggested approach is to enter on disk not the detailed biology which presently is on tape, but the summarized data elements which are generated for the various biology reports. At present, the summarization is obtained every time a report is generated; in the proposed approach, it would be carried out as the file is updated. Additional data elements, at present not on any report, may be added. A redesign of the biology data base will thus have to be carried out before online access can be implemented.

In our system, the input of chemical structures, particularly for queries, is unnecessarily complex. Querying the system requires a great deal of system-oriented knowledge, such as which keys to use and in what sequence, information which is conceptually irrelevant to the query. Consequently, few are the investigators that have taught themselves to use the system. Better query languages and input methods for structures have been developed, for example by Merck, by Upjohn, and for CAS ONLINE. An input method (which has been found patentable) has been developed in-house, which takes advantage of the capabilities of our Hewlett-Packard terminals. Either of these approaches, or a combination of them, could replace our present procedure, and allow users, without much training, to interact directly with the system. Further, if input entry were easier, and updating were performed online, the acquisitions contractor could make the initial entries, rather than CAS. The structures then would be immediately accessible

throughout the system, allowing users to determine, online, whether a compound was registered, had been shipped, or that an error was awaiting resolution. At present, such information reaches the system with a delay, often of weeks.

Currently, on our files, two sets of representations are carried for each chemical structure. One, the connection table, is what the machine examines when searching for ID or substructure; the other representation consists of vectors producing graphic displays. These require considerable storage. Two 3330 disk packs, each capable of storing 200 million characters, are presently required. We could obtain structures less demanding of storage, and requiring little additional computation, by adding one byte to each line of the connection tables. This would allow regeneration of the original structure, the extra byte indicating bond orientation. Assuming an average of 12 non-H atoms per structure, this additional storage requirement would amount to 3.6 million characters for our 300,000 compounds, or a savings in storage space of two orders of magnitude. The esthetic appeal of the output structures need not be impaired.

The system can further be improved by systematically eliminating inconsistencies and duplications. For example, at a recent meeting, in which CAS and IITRI participated, it became apparent : 1) that there was no feedback to inform IITRI of changes in the status of QNS compounds which they listed; 2) that IITRI is not aware that additional amounts may be available for some of the compounds they declare QNS; 3) that no mechanism exists for removing from a QNS listing a compound whose supplier was changed. Though this particular problem has since been resolved, there is little doubt that others remain.

Another example of an inconsistency is that, to perform logic on a query, a user must request a substructure search! To list compounds obtained, say, from source A, and of which at least 2 grams are in store, one must enter these specifications, together with the connective "AND". The user can enter the connective only by requesting the system to perform a chemical substructure search (which this certainly is not). Evidently, in the early stages of the system, only substructure searches required the logic AND.

As another example, manual requests for shipment of a sample are executed by the contractor (FLOW) before a corresponding transaction is entered into the system. The system then monitors for the receipt of the shipment by the screener. Had the transaction been entered at the time the request was made, the system could have monitored also for the timely execution of the shipment, avoiding embarrassing slippages. No additional input would have been required, and the system could have prepared shipping lists and labels, saving work. The present arrangement is apparently the result of the implementation of the inventory system two years following the implementation of the chemical system.

Finally, for about 10% of the file, more than one NSC number has been assigned to a chemical compound. This would happen when the same compound was submitted twice, once from an "open" source, once from a "discreet" one. Duplicate compounds no longer are accepted, but their presence in the file complicates processing and reporting.

Many more such instances could be cited. Although these could be corrected piecemeal, they could be taken care of, much more efficiently, within the context of a general system redesign. But can we afford a redesign under the present austere financial climate? The decision of whether to make the present system do for another few years, or whether to redesign it, in its entirety or in part, will be a difficult decision, a considerable challenge for the new Information Technology Branch.

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ANNUAL REPORT OF THE NATURAL PRODUCTS BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 - September 30, 1981

One of the major objectives of the Division of Cancer Treatment is to discover novel types of compounds with antineoplastic activity which can provide a basis for new chemotherapeutic agents.

In this regard natural product research has a long history of producing novel and unusual types of chemical structures which show many types of biological activity and indeed the basic structural types of various classes of natural products have led to development of many major classes of chemotherapeutic compounds. The investigation of natural substances for anticancer activity can provide new types of compounds for evaluation which possess unusual properties and can lead to new drug classes for chemotherapeutic testing in cancer treatment.

The Natural Products Branch has actively pursued acquisition, isolation, structure determination and testing of compounds from microbial, plant and animal sources in order to obtain new leads for further development in the NCI program.

The major program areas of the Natural Products Branch include: 1) contract research directed toward isolation of new agents from active extracts of plant, animal (including marine organisms) and microbial sources; 2) acquiring three types of human interferon (leucocyte, fibroblast and lymphoblastoid); 3) world-wide procurement of new plants, marine organisms and microbes for preparation of extracts and screening for antineoplastic activity; 4) procurement and preparation of large quantities of active agents for drug formulation, tumor panel testing, toxicology and clinical studies; 5) world-wide literature surveillance for the acquisition of natural products with demonstrated biological activity or novel structural types for evaluation.

Objectives

As part of the overall Division of Cancer Treatment Program, the Natural Products Branch has three major objectives:

I. Fermentation

The acquisition of new antineoplastic agents from microbial fermentations is the primary responsibility of this area. A contract program exists to isolate unique organisms and to ferment and prescreen for biological activity. Active fermentations in the prescreens are then tested in P388 leukemia in vivo. Active broths are assigned to chemists and then the active anticancer agent is isolated and evaluated by NCI. The capacity exists in these contracts to produce sufficient amounts of material for clinical studies. The program is using novel methods and unusual sub-

strates to obtain unique organisms in the hope of generating numerous new structural leads. In vitro prescreens (enzyme inhibition, tubulin binding, yeast and bacterial spectrums, phage induction and cytotoxicity vs various cell lines) have increased the efficiency of the program and decreased the in vivo testing load for primary fermentations.

II. Plant and Animal Products

The acquisition of new active antineoplastic agents from higher plants and from animal products (marine, primarily) is the major function of this section. A contract program exists to obtain, select and process enough plant material to obtain sufficient novel material for chemical identification and antineoplastic testing. Critical to this program is the ability to ensure a supply of plant material which is deemed of interest to NCI. This program is in need of in vitro prescreens to allow more quickly the isolation of new antineoplastic agents. A contract award has been made to develop three in vitro prescreens for testing plant and fermentation extracts.

A contract program exists to obtain marine animal products in sufficient quantity to extract, isolate and identify the active antineoplastic compounds. We are at present evaluating submerged fermentation of plant cells as an alternate route to supply plant products and to study the influence of precursors on yield improvement of the anticancer compound of interest. Maytansinoids and triptolide have been successfully produced in this manner. Work is underway to produce homoharringtonine by this method.

III. Worldwide Surveillance of Natural Products

This function is primarily done in the Office of the Chief. Literature, personal contacts with scientists, universities and industrial companies allow NCI to determine if any new natural products with various biological activities have been isolated and are worthy of testing in the National Cancer Program. Selected biological activities, new organ site specificity, or new structure determines whether a compound should be obtained and evaluated in NCI tumor screens. The literature surveillance contract has been highly effective in identifying potential new compounds of interest to NCI.

Program Implementation

The objectives of the Natural Products Branch are carried out through the Sections and the Office of the Chief and are:

I. Fermentation Section

The objective is the isolation and development of novel antineoplastic drugs derived from microbial fermentations. In support of these tasks the Section maintains four contracts to isolate unique organisms and to systematically evaluate the microbial world for its ability to produce novel anticancer agents. The Section maintains seven contracts to isolate, identify and scale up active materials of interest for NCI evaluation. These antineoplastic agents are supplied in quantities sufficient

for toxicology and clinical trials.

The Section directs two programs in biotransformation and co-metabolism. The work includes screening of microbes, fermentation, fermentation development, chemical isolation and genetic and culture research. More than 15,500 cultures were fermented this year and evaluated for their ability to produce novel antineoplastic agents. Two contracts are devoted to biotransformation of known structures and co-metabolism of various unusual compounds. Biotransformation is the microbial technique used to convert a compound to one of lower toxicity, broaden activity, increase solubility or make it pharmacologically more acceptable to man. A microbe can carry on as many as 20 chemical reactions, many at the same time, producing only one isomer and not destroying the primary structural moiety of a compound. This area seems to be quite productive and is being continued.

The use of microbes to produce antineoplastic agents has been used by NCI since 1956 and has been quite productive in producing compounds which go to clinical trial.

TABLE 1

FERMENTATION PROGRAM STATISTICS

Approximately 15,000 microbes isolated/year

- (a) 191,876 culture broths (primary) tested in vivo since inception of program
- (b) 15,480 confirmed actives since inception of program
- (c) 2,003 crystalline metabolites tested since inception of program
- (d) 203 new compounds in tumor panel (in 1980) 1/
- (e) 113 new compounds in special testing (in 1980) 2/

1/ Tumor panel consists of in vivo testing against five murine tumors and three xenografts (human tumors in nude mice) or renal capsule

2/ Special testing - against any of 1/

II. Plant and Animal Products Section

The objective is the development of novel anticancer drugs from higher plants and animals (including marine organisms). In support of this activity the Section maintains a world-wide program of acquisition of plant and animal materials. These materials are supplied by three collection contracts, purchase of new extracts and voluntary submissions from natural products researchers. A contract also exists to prepare extracts of new materials for preliminary screening. Materials corresponding to active extracts are then recollected in quantities large enough for fractionation studies. Fractionation is undertaken by one of four contractors who undertake the isolation, purification and structural elucidation of the active substances in the extracts. There

are three contractors involved in isolation of novel antineoplastic agents from plant materials and one contractor primarily involved with animal extracts. These contractors also reisolate larger quantities of pure compounds for further screening as required. A preparative scale-up isolation laboratory is available to process large amounts of plant, extract and produce substantial amounts of pure compound for toxicology, pharmacology and clinical studies. The use of plants to obtain antineoplastic agents has been done at NCI since 1957. The animal program was not formalized until 1970.

TABLE 2

PLANT PROGRAM STATISTICS

1500-3000 plant samples/year received by NCI

- (a) 500-900 different plants/year
- (b) Approximately 35,000 plant species and 113,000 extracts tested since inception of program
- (c) Plant extracts active - 4887 since inception of program
 - 1) 1549 genera
 - 2) 3386 species
- (d) 77 plant compounds in tumor panel (in 1980)
- (e) 16 plant compounds in special testing (in 1980)
- (f) 2372 crystalline plant compounds tested

TABLE 3

ANIMAL PROGRAM STATISTICS

3000 animal species extracted and tested since inception of program

- (a) 16,029 extracts tested since inception of program
- (b) 660 confirmed actives
- (c) 413 genera
- (d) 561 species
- (e) 590 crystalline animal compounds tested
- (f) 7 animal compounds in tumor panel
- (g) 10 animal compounds in special testing

III. Worldwide Surveillance

This activity is carried out by the Branch and is important in acquiring novel products for evaluation in the program. This maintenance of worldwide contacts with scientists, industrial companies or universities is essential in order that NCI may acquire as early as possible any novel compounds with interesting biological activity. These compounds are reviewed for interest based on structure, various biological activity and antitumor activity and if deemed interesting are tested in the NCI program. This type surveillance is important to determine what screens and what type compounds are being evaluated for cancer research worldwide. The literature surveillance contract and industrial, academic and research institutes have been valuable sources of new compounds.

Branch Staff

The Natural Products Branch is composed of a staff of six persons consisting of three senior professional scientists (GS 13 or above), one other professional staff member and a clerical staff of two.

The responsibility for the activity of the Branch is as follows:

Natural Products Branch

Chief - Dr. John D. Douros
Plant and Animal Products Section
Head - Dr. Matthew Suffness
Fermentation Section
Head - Amelia Acierto

Program Operation

The fermentation program has been designed to hopefully obtain more novel organisms and ferment these cultures under various conditions which should produce novel antineoplastic agents. Work continues to develop new prescreens (in vitro) in order to obtain new structures with antineoplastic activity. The prescreens allowed the major NCI fermentation contractors to evaluate 11,958 new fermentations. This is 3,000 less than last year due to 1 contractor having so many leads in chemistry. One thousand seven hundred thirty-four of these fermentation broths were active in one or more of the prescreens (antimetabolite, microbial screen, tubulin binding, antiviral, enzyme inhibition). Nine hundred fifty of the in vitro actives have been regrown and tested in vivo vs P388 leukemia in the mouse. Normally when all crudes were tested directly in vivo (until 1975) approximately 3% were active. Using the prescreen 15% of the in vitro actives were active in vivo. In addition, with presumptive chromatography now being done before the in vivo step most of the actives should be novel. We also found out in our experiment that concentrating the in vitro active broths allowed 42% to be in vivo active.

Results in Table 4 indicate a highly productive year even with a decreased budget. At present we have 203 microbially derived compounds in the tumor panel and 113 microbial materials in special testing. This year many novel

fermentations (110) with in vivo P388 activity were assigned. Due to the budgetary reductions we have had to cut our fermentation and chemical efforts which is the primary reason fewer materials were isolated. This number is still far above years prior to 1975.

TABLE 4

1980 Fermentation Statistics

<u>Company</u>	<u>Cultures Fermented</u>	<u>Active In Vitro</u>	<u>Tested In Vivo</u>	<u>Active In Vivo</u>	<u>Undergoing Chem. Fract'n</u>	<u>New Active Compounds</u>
A	880	140(16%)	125	55(44%)	90	7
B	1025	458(45%)	260	108(42%)	28	7
C	<u>10,053</u>	<u>1136(11%)</u>	<u>565</u>	<u>60(11%)</u>	<u>42</u>	<u>4</u>
Total	11,958	1734(14.5%)	950	143(15%)	160	18

Biotransformation and co-metabolism are two fermentation techniques being evaluated for their potential to produce interesting microbial metabolites (two contracts). The biotransformation and co-metabolism program is designed to modify compounds which have a structure nucleus of interest to NCI but the drug itself is either too toxic, has some ill-desired side effect, or is not quite active enough. It is hoped with subtle select modifications made microbially a superior compound will be obtained. Microbes are known to do approximately 20 chemical reactions and can do many of these at the same time on a compound without destroying the structure. Thirteen compounds were obtained from the biotransformation program this year and are being evaluated. Twenty-two compounds were exposed to this technique and in many cases more than 100 microbes were used in an attempt to get a modification of the structure. In addition, co-metabolism fermentations have been initiated. This is the feeding in of an unusual chemical in the hope of influencing the microbe's metabolism and having it produce new antineoplastic agents. Table 5 shows the submissions from this work.

TABLE 5

Biotransformation Submission (1980)

<u>Parent</u>	<u>Metabolite</u>	<u>NSC #</u>
Bouvardin	Bouvardin MB	328421
Mithramycin	Chromogenic aglycone	328422
Viridicatumtoxin	VA 188	328423
Mitomycin C	Ethylamine	329085

TABLE 5 (continued)

Biotransformation Submission

<u>Parent</u>	<u>Metabolite</u>	<u>NSC #</u>
Mitomycin C	Methylamine	329086
Mitomycin C	diff. methylamines	329087
Propargyl amine	Derivative A	331126
	Derivative B	331127
2-methylallyl amine	Derivative A	332583
	Derivative B	332584
N-propylamine	7-propylamino 7-desmethoxy Mitomycin B	333059
Aphidicolin	6B-hydroxy aphidicolin	340292
	18-acetoxyaphidicolin	339660

Table 12 shows the number of confirmed fermentation actives obtained in 1980 and Table 6 shows the total number of supposed novel fermentation leads being followed and will be assigned to chemists.

TABLE 6

Total Fermentation Leads as of 1981

	<u>T/C</u> <u>125-149</u>	<u>T/C</u> <u>150-174</u>	<u>T/C</u> <u>175-199</u>	<u>T/C</u> <u>200+</u>	<u>Total</u>
Bristol	0	2	1	3	6
Litton Bionetics	19	10	5	2	36
Upjohn	75	44	4	1	124
Warner-Lambert	<u>20</u> 114	<u>19</u> 75	<u>7</u> 17	<u>5</u> 11	<u>51</u> 217

Biological test systems are used to monitor the compound isolation; thus by the time the final product is isolated as many as 800 tests containing this material might have been evaluated. The amount of prior testing allows for greater insight into the handling and testing of the compounds and also a greater assuredness of the reproducibility of the material as a truly active compound. In some cases only the in vivo assay shows activity which makes

TABLE 7

Fermentation Contracts

<u>Contractors</u>	<u>Investigators</u>	<u>Contract No.</u>
Bristol Laboratories	Bradner	N01-CM-07299
Bristol Laboratories	Claridge	N01-CM-07324
British Columbia Univ.	Kutney	N01-CM-87236
Kyowa Hakko Kogyo Co.	Misawa	N01-CM-87190
Litton Bionetics (Chemotherapy Laboratory)	Flickinger	N01-C07-5380
Microbial Chemistry Research Foundation	Umezawa	N01-CM-57009
Warner-Lambert	Dion	N01-CM-07379
University of Iowa	Rosazza	N01-CM-07412
Upjohn Company	Neil	N01-CM-07380
University of Illinois*	Loub	N01-CM-97259

* Literature surveillance for all natural products

Kilogram quantities of daunomycin have been produced at our Litton Bionetics contractor. This contract is primarily a pilot plant research and development contract. Also, a successful Namalva lymphoblastoid interferon process has been developed. The crude interferon is being submitted to Dr. Anfinsen for purification and amino acid sequencing. Preliminary purification at FCRC using a hybridoma column has given 90% + pure interferon.

Several DN2 leads (largomycin, anticapsin, julimycin BII) are having processes developed for scale-up so we can produce sufficient material for the tumor panel. Large amounts of toyocamycin, an intermediate for the trinucleoside is being prepared. Two hundred grams of sangivamycin has been prepared for clinical use.

An IND on Ara-A was filed.

This year we obtained 38 pure antibiotic materials from our contractors and 154 from outside sources. A total of 113 fermentation antibiotics are undergoing special testing (in more than one animal tumor system) and 203 fermentation antibiotics are being tested in the entire tumor panel.

Plant and Animal Products Section

The operation of the plant products areas is divided into three main areas: procurement, preparation of extracts for screening and isolation studies of active extracts.

The initial procurement of plants comes from three major sources, an inter-agency agreement with the United States Department of Agriculture, a contract with the University of Hawaii and U.S. plants for aqueous extraction from Research Triangle Institute. Other plant samples are procured by purchase and voluntary submission from interested persons worldwide. Plant submissions are screened to eliminate excessive duplication of species already tested. When new areas are being explored, a list of botanical genera and species is reviewed and a selection made of what NCI wishes to have collected. The preparation of plant extracts for screening is performed under a service contract with Raltech Scientific Services, Inc.

Approximately 2% of the extracts tested show reproducible activity in P388 in vivo. A selection of P388 in vivo active plants is made as to which plants are to be collected in large quantities for assignment to NCI fractionator contracts for isolation of the active anticancer compound(s). Hopefully prescreens can be made available to test plant extracts so NCI can obtain new compounds and expedite isolation of these compounds for testing. A contract in this area has been obtained and several prescreens are being developed for screening.

At present the plants are selected for fractionation based on the level of in vivo activity and phytochemical knowledge of the constituents of the genera and species showing activity. Much of this chemical constituent data is obtained through a computer survey at the University of Illinois and is acquired on an individual genus basis.

Table 12 shows the plant extracts which had confirmed PS activity in 1980. Table 8 shows the total plant extracts considered of interest at this time.

TABLE 8

Plant Leads Undergoing Fractionation

	<u>T/C</u> <u>125-149</u>	<u>T/C</u> <u>150-174</u>	<u>T/C</u> <u>175-199</u>	<u>T/C</u> <u>200+</u>	<u>Total</u>
Arizona State Univ.	7	8	0	0	15
Purdue University	7	2	2	0	11
Research Triangle	23	10	3	1	37
Univ. of Illinois	<u>12</u> 49	<u>11</u> 31	<u>11</u> 16	<u>0</u> 1	<u>24</u> 87

This year 23 plant materials were authorized for assignment to the chemists for fractionation. Those plants selected for isolation studies are collected in 100-300 lb. quantities and sent to one of three active contract laboratories for fractionation. The progress of fractionation of the plant extracts is monitored by testing fractions in the PS in vivo, KB in vitro, PS in vitro and astorocytoma in vitro system and pursuing isolation studies only on the active portion of the fractionation materials. Fractionation is continued until the active materials are isolated in pure form. The purified materials are tested thoroughly and screening data is closely monitored by the Natural Products Branch staff. Structural studies are performed on active compounds by the fractionation contractors. The production of active novel compounds from this approach has been satisfactory. We feel the addition of prescreens will allow for the finding of more novel active compounds.

The animal products area is handled in a similar fashion to the plant program except also doing preparation of extracts of new organisms as well as fractionation. The main emphasis in this area is on marine organisms which is a relatively new area and should lead to discovery of novel active compounds unrelated to those from the plant or microbial fields. Initial compounds indicate that the marine area will indeed yield completely different compounds from those found in the terrestrial world.

In addition, two contracts have been awarded for the total production of 100 billion units of leucocyte interferon with a purity of at least 3×10^6 units/mg of protein. One award was made for fibroblast interferon (50 billion units) and one award for 25 billion units of lymphoblastoid interferon.

This year 50 pure plant compounds were isolated and submitted by contractors and 135 by outside sources. A total of 16 plant materials are undergoing special testing and 77 materials are in the tumor panel.

A total of 40 pure animal compounds were submitted this year by the contractor and 7 new compounds came from outside sources. A total of 10 animal compounds are in special testing and 7 are in the tumor panel. The first marine compound (Didemnin B) passed DN2.

Table 12 shows the number of new animal extracts which had confirmed activity this year.

TABLE 9

Active Marine Leads Assigned to Chemists

	<u>T/C</u> <u>125-149</u>	<u>T/C</u> <u>150-174</u>	<u>T/C</u> <u>175-199</u>	<u>T/C</u> <u>200+</u>	<u>Total</u>
Arizona State Univ.	31	28	6	1	66

TABLE 10

Plant and Animal Products Contractors

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Agriculture, U.S. Dept. of	Duke	Y01-CM-40001
Arizona State University	Pettit	N01-CM-97297
Arizona State University	Pettit	N01-CM-97262
Hawaii, University of	Sagawa	N01-CM-33747
Illinois, University of	Farnsworth	N01-CM-97295
Polysciences, Inc.	Boettner	N01-CM-07300
Purdue University	Cassady	N01-CM-97296
Raltech Scientific Services	Statz	N01-CM-87182
Research Triangle Institute	Wall	N01-CM-97261
Burroughs-Wellcome (interferon)	Finter	N01-CM-17489
Warner-Lambert (interferon)	Beardmore	N01-CM-07292
Meloy (interferon)	Verna	N01-CM-07378
Flow Laboratories (interferon)	Edy	N01-CM-07370

Worldwide Surveillance

An extensive liaison is maintained between the Branch and the pharmaceutical and chemical industries, grantees, academic institutions, research organizations and other government laboratories to maintain our knowledge of natural products research and development throughout the world. Also, this liaison allows us to obtain prime compounds soon after their discovery for evaluation in the NCI program (e.g. Macbecins I and II, Tetrocarcin, Neothramycin, Mazethramycin, THP, Mitomycin RR-150, Geldanomycin derivatives, etc.).

This year 296 new compounds were obtained because of this liaison, many of which are active and are being obtained in larger amounts for evaluation. Close contact is maintained with the suppliers in order to provide interchange of chemical and biological data which is essential to insure efficient use of material.

A 16-day international visit to Japan to visit the following organizations was made.

Fujisawa Pharmaceutical Co.
 Ajinomoto Co.
 Sanraku Ocean Co.
 Asahi Chemical Co.
 Japanese Foundation for Cancer Research
 Suntory
 Yamasa Shoyu Co.
 Kureha Chemical Co.
 Sumitoma Chemical Co.
 Yamanouchi Pharmaceutical Co.
 Zenyaku Kogyo Co.
 Kaken Chemical Co.
 Institute of Microbial Chemistry
 Kyowa Hakko Kogyo Co., Ltd.
 Kirin Brewery
 Yoshitoma Pharmaceutical Industry

Shionogi & Co.
 Dainippon Pharmaceutical Co.
 Otsuka Pharmaceutical Co.
 Tokushima Bunri University
 Daikin Kogyo Co.
 Takara Shuzo Co.
 Ishihara Sangyo Co.
 Nippon Kayaku Co.
 Sankyo Co.
 Nippon Chemico Co.
 Mochida Chemical Co.
 Toyo Jozo Co.
 Meiji Kaisha, Ltd.
 Fujizuki Pharmaceutical Co.
 Takeda Chemical Co.

Many natural products are obtained from all parts of the world and from all types of organizations. The following criteria are used to determine if NCI is interested in testing such materials or should request them:

1. Compound has some anticancer activity vs. any tumor, whether in NCI's system or not
2. A novel structure never evaluated in our program
3. Activity vs. yeast, fungi or certain bacteria
4. Antiviral activity (human, animal or bacterial)
5. Reported enzyme inhibition of proteases lipases, cAMP phosphodiesterase, aminopeptidase, transmethylese, etc.
6. Other biochemical or biological activity

Several interesting compounds have come from this work: aclacinomycin (now in U.S. clinical trials); lentinan (in Phase II studies in Japan); rapamycin (passed DN2); largomycin (passed DN2); pepleomycin (passed DN2); bestatin (in clinical trials in Japan); neothramycin (in clinical trials in Japan); THP (in clinical trials in Japan) also krestin and picibanil, which are clinically used drugs in Japan.

Miscellaneous

This year one IND was filed on a natural product and four compounds passed DN2.

TABLE 11

ARA-A

NSC 404241

Accomplishments

The Natural Products Branch provided many new compounds to the program (Table 12). These materials were of fermentation, plant and animal origin. Four compounds have passed DN2 and are candidates for preclinical toxicology (Table 13).

Of the 424 pure natural products added to our program, 128 (30.1%) were obtained from contracts and the remainder were obtained through worldwide surveillance.

The Branch manages a total of 23 contracts with industrial, academic and research institutions, both domestic and foreign.

In summary, the following was achieved in the last twelve months by the Natural Products Branch:

1. A total of 5,382 new substances plus 424 pure compounds were obtained for screening:
 - a. 1,249 fermentation products
 - b. 503 animal products
 - c. 3,630 plant products
2. A total of 128 compounds were isolated and purified under contract:
 - a. 38 fermentation products
 - b. 50 plant products
 - c. 40 animal products
3. A total of 296 compounds were obtained from worldwide surveillance:
 - a. 154 fermentation products
 - b. 135 plant products
 - c. 7 animal products
4. Four new substances were assigned to Decision Network 2A:
 - a. 3 fermentation products
 - b. 1 animal product
5. Twenty-three contracts were supervised by the Branch (Table 7 and Table 10):
 - a. 7 fermentation
 - b. 2 biotransformation
 - c. 3 plant collectors
 - d. 3 plant fractionators
 - e. 1 plant extraction
 - f. 1 plant prep lab
 - g. 1 animal fractionator
 - h. 1 literature surveillance
 - i. 4 interferon (leucocyte)

TABLE 13

COMPOUNDS PASSING DECISION NETWORK 2A

7-Omen	NSC 269148
CC-1065	NSC 298223
Pepleomycin	NSC 276382
Didemnin B	NSC 325319

The Natural Products is involved in many analog committees. Dr. Douros chairs the Bleomycin, Actinomycin and Antimetabolite analog committees. Dr. Suffness chairs the Mitotic Inhibitors Committee.

During the past year the Natural Products Branch has assumed the responsibilities of evaluating natural products grant applications.

Major Accomplishments for Natural Products This Year

The major accomplishments were:

1. Three fermentation products (aclacinomycin, pentostatin, acivicin) entered Phase II clinical trials this year and ARA-A entered Phase I.
2. Indicine-N-oxide and bruceantin - these plant-derived compounds are in Phase II clinical trials.
3. Homoharringtonine, taxol and echinomycin are in toxicological evaluation.
4. 120 grams of homoharringtonine was obtained from the Chinese. This is enough to complete toxicology and Phase I clinical trials.
5. Several kilograms of daunomycin were produced at the Frederick Cancer Research Center (FCRC).
6. We obtained 424 new natural products for evaluation. 128 of these were from contractors.
7. The lymphoblastoid interferon process continues to be improved, costs decreased and purification optimization continues in order to produce clinical grade interferon. All the interferon procurement contracts had been let on leucocyte, fibroblast and lymphoblastoid interferon.
8. 203 fermentation, 77 plant and 7 animal compounds are in the tumor panel.
9. A successful Natural Products Contractors Meeting was held in Kalamazoo.
10. More than 11,000 cultures were fermented and prescreened this year.
11. 4 kilos of Indicine-N-oxide was prepared in the plant pilot plant.
12. 13 pure biotransformation products were submitted this year.

13. 200 grams of sangivamycin were produced for clinical evaluation.
14. 5 grams of largomycin were prepared.
15. The toyocamycin fermentation was optimized for large tank fermentations

TABLE 12

MATERIALS ACCESSIONED AND ACTIVITY REPORT

	Extracts Submitted		No. Extracts Active <u>in vivo</u>		Crystalline Material Submitted		Dec. PT. II ^a		INDS filed	
	<u>1979</u>	<u>1980</u>	<u>1979</u>	<u>1980</u>	<u>1979</u>	<u>1980</u>	<u>1979</u>	<u>1980</u>	<u>1979</u>	<u>1980</u>
Fermentation Products	1249	879	73	92	285	182	0	3	3	1
Animal Products	503	1102	2	7	50	47	0	1	0	0
Plant Products	3630	4872	80	56	187	185	1	0	0	0

^a Selected candidates for pre-clinical toxicology evaluation and clinical trials

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ANNUAL REPORT OF THE ANIMAL GENETICS & PRODUCTION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 to September 30, 1981

The primary function of the Animal Genetics and Production Branch (AG&PB), Developmental Therapeutics Program (DTP), Division of Cancer Treatment (DCT), is to provide healthy laboratory animals with properly defined genetic characteristics to various research investigators as follows: (1) DTP Screening Program; (2) other NCI Research Contracts (DCCP, DCBD, etc.); (3) NIH Intramural Program; (4) Research Grants; (5) NIEHS, and (6) Veterans Administration Research Facilities (surplus animals which are offered when available).

An additional function of this Branch is to coordinate the movement of transplantable experimental animal tumors from tumor bank sources into scientific laboratories.

The structure of the animal production program includes: 4 Primary Genetic Centers; 7 Rodent Production Centers; 10 Hybrid Production Contracts; 2 Beagle Hound Production Contracts; 7 Animal Disease Diagnostic Contracts; and 1 contract with the National Academy of Sciences. FY 1981 funding for the efforts listed above is included in this report.

The Branch staffing consists of one senior staff member, one professional associate, one animal contracts assistant, and one clerk typist. Contracts and Branch functions are currently structured as follows:

PRIMARY GENETIC CENTERS

These centers serve as a breeding nucleus for the animal program. In order to insure that maximum quality control is maintained at this level, it is required that any animals received by such centers are derived into a germfree state, i.e., Caesarian sections are performed under isolator conditions. These animals are then maintained as foundation colonies in defined flora isolators. These foundation isolators serve as a source of breeders for the larger expansion colonies which are housed under barrier conditions.

RODENT PRODUCTION CENTERS

The purpose of these contracts is to accomplish large-scale production of pure strains of inbred mice which will be supplied to fixed price contractors for hybrid mouse production. The rodent production contracts receive breeding stock from the genetic expansion colonies. These breeders, therefore, represent direct offspring of brother x sister matings. This production also takes place under rigid barrier-controlled conditions. These contracts are essential for providing the large number of "super clean" breeders for hybrid production contracts.

HYBRID MOUSE PRODUCTION CONTRACTS (Fixed-Price)

These contractors supply the large volume of first generation hybrid mice for the screening program. Breeding animals for these colonies are received from the rodent production contracts.

BEAGLE PRODUCTION CONTRACTS

These animals are produced on a fixed-price contract basis from qualified breeders and are primarily used by the toxicology program.

DIAGNOSTIC CONTRACTS

These are service contracts which are utilized to monitor and upgrade the quality of animals moving within the animal production program and to the laboratory. Presently, they consist of the virus diagnostic services, three pathology services, one bacterial diagnostic service (primarily Salmonella and Pseudomonas species), and one isolator monitoring (associated flora) contract.

HISTOCOMPATIBILITY STUDY CONTRACTS

These contractors perform genetic monitoring of inbred mice and rats by means of skin grafts.

TUMOR BANK CONTRACT

This contract provides experimental human and animal tumors (free of contaminating microbes) on a scheduled basis to screening/tumor panel laboratories and to qualified investigators both in the U.S.A. and abroad. Due to an increasing awareness of genetic drift problems, a number of investigative laboratories have asked to be placed on the screening schedules, thus, increasing the workload of this contract.

NATIONAL ACADEMY OF SCIENCES

A small amount of support is supplied to the National Academy of Sciences. This support involves development and publication of standards and guidelines in the field of animal care and breeding. The goal of this Branch is to achieve and maintain quality animal production while meeting the needs of the program.

The cooperation of the Frederick Cancer Research Center (FCRC) again merits special recognition. The cooperative effort between DCT and FCRC enabled FCRC to remain afloat (interim breeder animals from DCT genetic centers) until re-derived animals were expanded in sufficient numbers to meet FCRC needs. The rapid expansion of nude mouse production at FCRC enabled the AG&PB to meet DCT's critical needs for human tumor screening on schedule. Quality of animal production at FCRC continues to be equal or superior to any production facility in the U.S.A.

The reimbursement program provides a service to other NCI divisions, intramural investigators and to grantees since animals of the quality and quantity needed are not available with the NIH background. DCT benefits from the reimbursement program because a significant percentage of the animals supplied to other

programs are surplus to DCT's needs (offsex, etc.), thus, providing a new savings to the DCT Animal Program Budget. The savings provided to the taxpayer by avoiding duplication of effort in laboratory animal production, and genetic and health monitoring is also obviously significant.

The DCT/NCI Program is now in a position to provide "super clean" animals to those facilities that can maintain these animals in a comparatively disease-free condition. Special programs that necessarily require this quality level include human tumor testing, Biological Response Modifiers, NCI tumor bank, NCI intramural laboratories, etc. A number of testing laboratories are upgrading their facilities in order to receive and hold these animals, e.g., Southern Research Institute, Arthur D. Little, Inc., Roswell Park, Yale University, etc. Within the limits of budgetary considerations, it would appear appropriate to encourage further upgrading of testing facilities with the consequent elimination of animal disease variables in cancer research data.

ANIMAL GENETICS & PRODUCTION BRANCH PROGRAM FUNDING

FY 1981

<u>PRIMARY GENETIC CENTERS (4)*</u>	<u>\$4,307,000</u>
Supply breeding nucleus for the animal program and athymic mice for drug evaluation.	
<u>RODENT PRODUCTION CENTERS (7)*</u>	<u>1,813,000</u>
Large-scale production of inbred mice under both conventional and barrier controlled environment.	
<u>HYBRID MOUSE PRODUCTION CENTERS (10)*</u>	<u>2,213,000</u>
Supply hybrid mice for the screening program.	
<u>BEAGLE PRODUCTION CENTERS (2)*</u>	<u>60,000</u>
Supply dogs for large-scale toxicology.	
<u>DIAGNOSTIC AND HISTOCOMPATIBILITY PROJECTS (7)*</u>	<u>859,000</u>
To monitor animal health and genetic integrity.	
<u>DEVELOPMENT OF STANDARDS AND GUIDELINES (1)*</u>	<u>27,000</u>
For animal care and breeding.	
<u>MAINTENANCE OF FROZEN TUMOR BANK (1)*</u>	<u>199,000</u>
<u>PRODUCTION OF ATHYMIC AND OTHER NEEDED MICE (FCRC)</u>	<u>367,000</u>

*Number of Contracts

TOTAL	<u>\$9,845,000</u>
Less Reimbursements	<u>1,545,000</u>
COST TO DCT	<u>\$8,300,000</u>

ANNUAL REPORT OF THE DRUG EVALUATION BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 - September 31, 1981

I. Objectives

Objectives of the Drug Evaluation Branch are the recognition of new agents for development to clinical trial against cancer and the conduct of pre-clinical therapy related tasks essential or complementary to new anticancer drug development. In achieving objectives, DEB plans, directs and evaluates a comprehensive contract program to design, implement, maintain, and coordinate laboratory projects for screening *de novo*, large numbers of synthetic and natural products for biological activity indicative of clinical utility, in-depth evaluation of materials of interest found in initial screens conducted by DEB or reported from other programs, and development of improved methods for pre-clinical drug evaluation. Principal end-products are recommendations to the Associate Director, DTP and Director, DCT of new drugs for development to clinical trial, pre-clinical therapeutic data required to initiate clinical trials, reports on pre-clinical efficacy for inclusion into Investigational New Drug Applications to the U. S. Food and Drug Administration, communication of pertinent findings to clinical cooperative groups and the scientific community via presentations in meetings and symposia and publication in scientific literature.

II. Implementation

Program implementation is through contracts with qualified institutions. As of April 30, 1981, DEB directed 19 contracts funded at approximately \$10,000,000 (Table 1). The workscope of each contract is summarized in the Report of the Director, DCT.

III. Organization

Significant organizational changes were accomplished or were expected to be finalized during FY 1981. The Mammalian Genetics and Animal Production Section was elevated to Branch status consistent with the Agency wide impact of its services. The Automated Information Section, DEB and the Chemical and Drug Information Section, DS&CB were transferred to the newly established Information Technology Branch (ITB) to consolidate biological and chemical information resources and to increase functional and fiscal efficiency in their utilization. Changes within DEB (in process, April 1981) include abolition of the Screening Section and establishment of three Sections: The Screening Operations Section, The Selected Agents Section, and the Cell Culture Section. The Section Reports for this reporting period (appended) do not reflect these recent changes.

IV. Staff Functions

As of April 30, 1981, DEB staff included eight senior professionals, two junior professionals, and five technical and clerical personnel (excluding four professionals and three technical personnel reassigned as a result of DTP reorganization during the year). The addition of one Cancer Expert was in process.

Staff functions to (a) determine scientific approaches to realization of mission objectives; (b) devise appropriate experimental protocols; (c) implement Program by generating project plans, requests for proposals, and contract summaries; (d) review proposals and participate in source selection; (e) serve as contract Project Officers to insure scientific and technical sufficiency of contractor performance; (f) assimilate, review, and summarize laboratory testing results; (g) present potentially improved therapies against human cancer to the Associate Director, DTP, DCT decision groups, and clinical groups; (h) present major findings to the scientific community through publications and presentations at professional meetings; (i) prepare reports of pre-clinical therapeutic efficacy of new drugs for inclusion in Investigational New Drug Applications (INDA) and clinical brochures; (j) review grant applications for relevancy to DCT objectives; and (k) advise DCT program staff on approaches to realization of goals.

Staff collaborates actively with other DTP Branches to assure maximal efficiency of resource utilization in carrying out their interdependent functions. During 1981 DEB senior personnel presented a report on progress of the screening program to the DCT Board of Scientific Counselors and served on the committees and working groups listed in Table 2.

Between April 1, 1980 and March 31, 1981 DEB summarized the pre-clinical therapeutic data for Tricyclic Nucleoside Phosphate (NSC-280594) and updated a similar report for 5-methyl tetrahydrohomofolate (NSC-139490). The reports were submitted to the Cancer Therapy Evaluation Branch, DCT for inclusion in the IND Applications to the U.S. Food and Drug Administration.

Approximately 125 grant applications dealing with all aspects of drug development except synthesis of new agents and isolation of natural products were reviewed. Three types of evaluation were provided: (1) factual or technical information, such as the status of a particular compound in drug development; (2) Program relevance of each application and its possible overlap with contract supported activities; and (3) the priority of each application as high, medium, or low in relation to the objectives of DCT. The information was supplied to the Grants Program Director, Office of Extramural Research and Resources, to the Associate Director, DTP, and to the Director, DCT to aid in the review of grant applications, to determine the extent to which grant studies complement contract projects and to assist DCT in decisions regarding the funding of projects with similar priority scores.

V. Screening for Antitumor Activity

Figure 1 depicts the current flow of drugs through DCT screens and shows the addition of human tumor stem cell cloning assays to the overall plan. Except for this addition, current screening procedures are substantially the same as those described previously and are summarized in the report of the Screening Section.

A. In Vivo Screening

During this reporting period (April 1, 1980 through March 31, 1981 a total of 22,483 materials (13,799 synthetic agents and 8,684 crude natural products) were screened for the first time. The numbers of tests conducted in each host-tumor model comprising the DCT Pre-clinical Tumor Panel are in the current Report of the Screening Section. As of March 31, 1981 a total of 1,828 compounds had been selected for tumor panel testing, 252 during this period. Of the latter, 200 entered on the basis of confirmed activity in the leukemia P388 in vivo pre-screen; 46 "bypassed" the P388 pre-screen on the basis of known biological or biochemical action or the rationale for synthesis. Six were recommended by DCT Analog Committees. The Pre-screen and/or Data Review Subcommittees of the Drug Evaluation Committee (DEC) reviewed screening data for 1,566 materials, referring 49 with selected analogs to the DEC. The DEC reviewed 72 compounds including Subcommittee referrals, Analog Committee referrals, and compounds with data from non-Program sources. Materials meeting established criteria for Decision Network (DN) 2A acceptance were referred to the Associate Director, DTP.

Twenty-three materials passed Decision Point 2A or higher in the DCT Linear Array. Of these, 16 were recommended by the DEC or analog working groups on the basis of screening activity, one was selected on the basis of clinical reports and screening activity, four had been selected in prior periods and were resurrected because of renewed clinical interest, and three were analog replacements of materials selected previously. Materials that passed DN2A during this period are listed in the current Report of the Screening Section, DEB.

The following discussion of the status of the DCT Tumor Panel was based on a management analysis (Part B) current through March 23, 1981 and a scientific analysis (Part C) presented to the DCT Board of Scientific Counselors on February 12 and 13, 1981. Because of the differences in the times of these analyses, their data bases (numbers of compounds, etc.) will vary from that reported above which covers the period through March 31, 1981.

B. Tumor Panel, Testing Status

The mouse tumor assays used for in vivo pre-screening and DCT Tumor Panel (TP) testing are listed in Figure 2. The status of TP

testing as of March 23, 1981 is summarized in Tables 3-5. One thousand eight hundred and seventeen compounds had been entered into the TP, but 105 had been dropped by Program prior to completion of testing. Of 1,712 remaining compounds, 671 (39%) were completed in all eight of the TP models, 406 between April 1, 1980 and March 23, 1981 (Table 3). However, inasmuch as many others had been completed in most of the systems, the completion rate was substantially greater for each individual system (Table 4). For example, the completion rate ranged from 77-81% for mouse tumor models and from 46-58% for human tumor xenografts. Approximately 16% of compounds designated for TP testing were not available in sufficient amount for one or more test systems. The remaining compounds, about 7% scheduled for mouse tumor testing and 23-35% for xenograft testing, were "on test" or awaiting testing (Table 4). Elimination of those materials not available for testing (Table 5) shows that the completion rate was greater than 90% for each of the mouse tumor models, and the completion rate for human tumor xenografts ranged from 57% for the mammary tumor, MX-1, to 72% for the colon tumor, CX-1 (Table 5). The lower completion rate for xenograft testing was a consequence of delays in reaching full testing capacity described in earlier reports. This is reflected in Table 6 which shows, for each model, the cumulative completion rate through each of the last three reporting periods. On March 21, 1979, when the total number of TP compounds was 1,200, the completion rate for mouse tumor models ranged from 53% (CD8F₁ mammary carcinoma) to 78% (L1210), but the completion rate for xenografts ranged from 8-11%. During 1979 and 1980 earlier problems with the CD8F₁ and Lewis lung tumors were overcome, and as of March 23, 1981, the differences in completion rates among animal tumor models were minimal. With the xenografts, the contrast between the very low completion rates through March 31, 1979 and the relatively high rates for later periods is attributable to the increased availability of athymic mice and the change from the subcutaneous tumor site to the renal capsular site. The lower rate of completion for the MX-1 xenograft relative to other xenografts is a consequence of the greater sensitivity of the MX-1 tumor, the need to retest active materials in order to complete testing, and the need to use some capacity to test the intralaboratory and interlaboratory producibility of the MX-1 model.

It is estimated that we will need to complete the testing of 2,000 compounds in order to properly evaluate the TP as a pre-clinical experiment. Progress over the last 2-3 years has been steady. The four laboratories engaged in xenograft testing (Battelle Memorial Institute, 0-7099; Mason Research Institute, 9-7317; IIT Research Institute, 9-7316; and the Southern Research Institute, 9-7309) have a current total test capacity to evaluate 625 compounds per year in each of the three xenograft models. Pragmatically, however, we can expect the existing test capacity to provide for the completion of approximately 400 compounds per year based on last year's actual completion rate of 406 (Table 3). The capacity to test is consistently greater than the actual testing rate because of a variety of factors including invalid tests due to sick mice, technician errors,

contaminated tumors, etc.; repeat testing of analogs in direct comparison with the parent compound; and the need to conduct studies of test reproducibility for statistical analysis. Thus, we can expect to complete 400 compounds per year in the TP - the rate of xenograft testing is the limiting factor related to the models per se - if the current level of effort is maintained and if the supply of healthy athymic mice continues at its present rate. With the introduction of the renal capsule xenograft technique in early 1980 and an improved computerized management system for tracking compounds assigned to the TP, we have been able to provide better balance to the screening program. Emphasis over the last year has been on the initiation of more xenograft testing to reduce backlogs and more P388 testing to decrease the time required to obtain pre-screening results. In order to affect this balance, we have reduced the testing rate in some mouse tumor models such as L1210 and B16 (Table 7). Table 8 illustrates the amount of testing and time required to reach our goal of 2,000 compounds completed in all TP models if resources permit maintenance of a completion rate of 400 compounds per year. There is little question that testing against human tumor xenografts required considerably greater effort than testing against mouse tumors. Although the change from the subcutaneous tumor assay to the renal capsule assay reduced xenograft testing costs by 40%, a xenograft test (renal capsule) is, nevertheless 2-6 times more costly than a mouse tumor test (Table 9).

C. Tumor Panel, Analysis

In February 1981, a preliminary analysis of TP results was presented to the DCT Board of Scientific Counselors. The analysis was based on 588 compounds completed in all models at that time. Table 10 summarizes, for each test system, the percentage of compounds meeting MC-1 and DN2 activity criteria. The MC-1 activity level is indicative of statistical significance. The DN2 activity level is sufficient to warrant consideration for presentation to the DCT Decision Network Committee as potential candidates for further development to clinical trial. DN2 activity criteria are substantially higher than MC-1 activity criteria. Of the 588 compounds analyzed, approximately 90% showed MC-1 activity or greater in the P388 pre-screen; 28% were active at the DN2 level. This is not surprising inasmuch as most of the 588 compounds entered the TP on the basis of activity in the P388 pre-screen. Analysis of DN2 actives in the eight TP models shows that the mouse and human lung and colon tumors were relatively refractory to treatment, the mammary tumors were moderately sensitive; and B16 melanoma and L1210 were the most sensitive of the tumor panel models (Table 10). Despite the preliminary nature of the analysis, it did permit tentative answers to critical questions asked at the inception of the TP (Tables 11-13).

Is the tumor panel selecting agents that would have been missed by screening with L1210 leukemia alone (Table 11)? Of 458 com-

pounds which failed to meet DN2 activity criteria against L1210, 103 elicited DN2 activity in another TP model. Table 11 lists the number of compounds showing DN2 activity against each of the TP solid tumors and failing to show DN2 activity against L1210. The total for the seven solid tumors is greater than 103 because some compounds were active in more than one tumor. Thus, the solid tumors in the panel are selecting compounds missed by L1210.

Are the human tumor xenografts and murine tumors selecting the same or different drugs (Table 12)? Of 89 compounds with DN2 activity in at least one xenograft model, 18 failed to demonstrate DN2 activity against a mouse tumor. Thus, human tumor xenografts are selecting some compounds that would not have been selected by a mouse tumor model.

Are human tumor xenografts and mouse tumors of a specific organ system selecting the same drug (Table 13)? A comparison of xenograft and mouse tumor responsiveness for colon, lung, and mammary tumors suggests little positive correlation based on histological type. For example, of 97 compounds active against the mammary human tumor or mammary mouse tumor, 47 were active against the human tumor only, 26 against the mouse tumor only, and 24 against both. The same pattern of lack of positive correlation was observed with the generally less responsive colon and lung tumor pairs.

From the inception of the TP in 1976 through calendar year 1980, 65 new compounds entered the DCT Decision Network Linear Array; 50 on the basis of activity against one or more of the TP models. Of the 54 compounds in development to the clinic as of March 23, 1981 (Operating Committee compounds), TP evaluation had been completed for 44. Of the remaining, three were not appropriate for TP testing, two were QNS, and the rest were in the process of completion.

DEB routinely bioassays experimental and final clinical formulations of drugs in development to assure retention of antitumor efficacy. Consistent with changing procedures for toxicological studies of new drugs, protocols for routine treatment schedule dependency studies were reduced from eight schedules to three. However, the effectiveness of intraperitoneal, intravenous, and oral drug administration is examined for all drugs in development to the clinic.

D. In Vitro Screening

Current in vitro screening can be divided into two activities: screening and bioassay in support of the Natural Products Branch (NPB) fractionation program and presumptive screening in human tumor stem cell cloning assays. We are anticipating the use of in vitro models for a third purpose, as pre-screens for the selection of crude natural products for screening.

1. Conventional In Vitro Screening and Bioassay of Natural Products

During this reporting period, crude natural products were tested in vitro by Arthur D. Little, Inc. (ADL, 9-7288) emphasizing the use of a mouse astrocytoma in culture. The astrocytoma was selected as the primary in vitro screen for crude natural products because, although it appears to be as sensitive to overall cytotoxicity as the L1210, P388, or KB cell cultures used previously, it is exquisitely sensitive to tubulin binding agents. All crude natural products are tested also in the P388 in vivo pre-screen. The reason for testing crude materials both in vivo and in vitro stems from the need for DEB to bioassay fractions of active materials during the course of fractionating leading to the concentration and isolation of the active component. Thus, when crude materials show correlative in vitro and in vivo activity, the less expensive and faster in vitro methods are used to bioassay the fractions; an activity conducted mainly by the University of Miami (9-7290). Fractionation products are tested in vivo periodically to assure that the cytotoxic fractions are also the in vivo active fractions. For the most part, in vitro testing of fractions was against KB, L1210, or P388 cell lines because the materials under fractionation were previously identified as active against these lines when tested as crudes. This aspect of cell culture testing is devoted almost entirely to support the natural product development program of the Natural Products Branch, DTP. During this reporting period, the two contractors conducting this work provided a screening capacity of 14,009 KB equivalents, defined as 3-5 concentration response from which the ED50 is interpolated.

2. Screening Against Primary Human Tumors in Culture; Human Tumor Stem Cell Cloning Assays (HTSCCA)

During the past year four contracts (Mayo Clinic, 0-7419; UCLA, 0-7420; University of Arizona, 1-7497; and the Cancer Therapy and Research Foundation of South Texas, 0-7327) were awarded for the adaptation and use of HTSCCA for screening. The assay is based on the system described by Hamburger and Salmon (Science 197: 461-463, 1977 and J. Clin. Invest. 60: 846-854, 1977). Single cell suspensions are prepared from primary human solid tumors, bone marrow effusions or pleural or ascitic fluids. The cells are incubated with test drugs (continuous exposure) in a two layer soft agar system for 7-21 days (Figure 3). Results are expressed as the percentage of control colony formation. Percentage responders per histological tumor type is the principal parameter of drug activity. The HTSCCA is currently in a pilot phase of development. The four laboratories are testing the same 50 drugs supplied blinded. In each laboratory, each drug is tested against a panel of ten human tumors representing 3-5 different histological types including at least four tumors of one histological type (Figure 4). If a material is active (cytotoxic) against a tumor it is subsequently tested against five additional tumors of that type. Our data processing contractor,

VSE Corp. (0-7251) is developing programs to process the data. FAS II image analyzers (Bausch and Lomb) for automated colony counting have been installed in the four contract laboratories. The current experimental testing protocol was developed at a DEB sponsored workshop in San Antonio, TX; December 1980. DEB staff in collaboration with other NCI staff, Pathologists, and Oncologists developed a preliminary scheme for classifying tumors based on the "Internal Classification of Diseases for Oncology" (WHO). The pilot study to be completed within a year is designed to answer key questions relative to the adaptation of the method to large-scale screening and also to conduct needed developmental work related to methodology (Figure 5). Mass screening will begin following completion of the pilot phase. The current screening plan is to test 1,000 compounds per year using the general protocol depicted in Figure 4. Two thirds of the input will be compounds that were inactive in the P388 in vivo pre-screen in order to uncover potentially active agents that may have been missed by our in vivo screening methods. VSE Corp. (0-7251) is currently developing an on-line conversational system to process data input and retrieval (Figure 6). Information will be transmitted via direct terminal between each contract laboratory and DCRT. An important feature of this arrangement is the ability to inform each laboratory regarding the drug(s) required to be tested against a tumor on the basis of initial diagnostic impression immediately when the laboratory receives the tumor from the operating room. The program also provides for subsequent modification of initial diagnosis when the pathology report is received.

3. In Vitro Pre-screens for Crude Natural Products

The high yield of antitumor effective antibiotics from in vivo screens relative to the yield of plant and animal products can be attributed in large measure to the use of diverse cell culture, microbial, and biochemical assays as pre-screens to select fermentations for screening. It is not necessary to impart any specific correlative activity to explain the success of in vitro pre-screens. By eliminating materials devoid of actions on biological systems, they reduce substantially the number of materials requiring in vivo testing and thereby increase the efficiency of the latter.

Historically, the yield of minimally active materials tested de novo in the P388 in vivo pre-screen has been about 5%. The yield of in vivo actives from crude fermentations selected on the basis of in vitro activity has been consistently about 16% (Table 14). At present Arthur D. Little, Inc. (0-7331, Natural Products Branch) is investigating a number of in vitro assays as potential pre-screens for crude plant extracts (Table 15). Within this year we plan to begin using at least one of the assays, probably the beta galactosidase phage induction test.

VI. Detailed Drug Evaluation and Model Development

A. Introduction

DEB manages multifaceted projects designed to provide maximal flexibility for rapid conduct of Program-directed exploratory, developmental, and applied studies pertinent to all pre-clinical therapy related tasks. Tasks are designed to (1) apply fundamental biological principles to the development of new and improved laboratory models as tools for the discovery of more effective anticancer therapies; (2) evaluate, in detail, drugs in development to NCI sponsored clinical trial; (3) conduct non-routine studies in response to DCT Program needs; (4) determine host-tumor histocompatibility in appropriate inbred and hybrid hosts; (5) describe biological growth characteristics including qualitative and quantitative aspects of metastases; (6) describe kinetics of growth of primary tumors and metastases; (7) determine, quantitatively, responses to treatment with clinically effective and ineffective drugs; (8) define the operational characteristics of the host-tumor system when used as a screen; (9) determine test-to-test reproducibility and variability; (10) recommend new host-tumor systems as models for (a) initial screening, (b) secondary or panel screening, (c) specialized screening including comparative testing of "analogs", (d) detailed drug evaluation including treatment route and schedule dependency, combination chemotherapy, and/or evaluation of combined treatment modalities; (11) provide therapeutic trial data relative to clinical predictive value compared with or contrasted to existing screens; (12) provide precise and detailed laboratory protocols for screening including parameters and criteria for activity; (13) provide estimated costs per test should the model(s) be utilized (in other laboratories) for general or specialized screening; (14) consult with other DCT contractors who may be asked to use the model(s); (15) develop preliminary information on the mechanism of action of new agents under development; and (16) elucidate biological, biochemical, and pharmacological factors which determine success or failure of treatment.

In order to maintain a vigorous and progressive drug development program, we have supported in vivo model development projects. Although considerable development work has been supported by NCI through the investigator initiated grant mechanism, few investigators tailor their studies to the special problems associated with large-scale drug screening. For example, few grant supported researchers develop a drug testing protocol or fully characterize systems so that evaluation criteria and control limits can be established. Because of our past and present support of developmental studies, we were able to fully implement the DCT tumor panel including human tumor xenografts and the renal capsule assay which has made large-scale xenograft testing feasible. This area has experienced severe cut-backs over the last several years.

B. Significant Findings

1. The Southern Research Institute (9-7309)

The Southern Research Institute (SRI) has conducted studies related to all of the tasks described in the above narrative although information on mechanisms of drug action is obtained indirectly from studies of cross-resistance. The contractor meets high standards for reliability and has gained an international reputation for their studies involving the biology of animal tumors and pre-clinical drug evaluation. Because of their strong commitment to model development, they have assisted our Program in developing many of the tumor panel models into systems suitable for mass screening of new agents. They have developed a number of transplantable murine colon and breast tumors with different properties, such as different metastatic potential, growth rates and drug responsiveness. Recently they characterized a murine model for pancreatic cancer and one for AML (RFM model). They assisted our Program by providing data on the potential of using cell kill as an endpoint in screening experiments. Current studies of special interest are: (1) a comparison of the stem cell in vitro assay and the renal capsule in vivo assay using a variety of murine colon tumors; (2) quality control studies (P388 and L1210 titration studies using mice from different suppliers); (3) an evaluation of the effect of caloric restriction on the growth of tumors implanted under the renal capsule in nude mice; and (4) a comparative study of the biological properties and drug responsiveness of the B16 melanoma line used in drug screening and the highly metastatic F10 line developed by J. Fidler. The accomplishments of this multifaceted project are too numerous to detail in this report and can be found in their comprehensive Annual Report of April 1, 1981. The following is a list of tasks completed and ongoing during this reporting period.

- a. Summary of screening experience with human tumor xenografts in nude, athymic mice.
- b. Cell culture of a 3-Methylcholanthrene-induced pancreatic tumor.
- c. Development of stem cell assay systems using transplanted murine adenocarcinoma colon tumors.
- d. Sensitivity of cultured cell populations derived from murine colon adenocarcinoma 26 and 38 tumors to selected antitumor agents.
- e. A new triazine antifolate (NSC-127755).
- f. Single-agent treatment of ten transplantable colon tumors of mice.

- g. Chemotherapy of the subcutaneous ovarian tumor M5076: loss of tumor response to MeCCNU.
- h. New murine leukemia model systems.
- i. Laboratory methods for the detection and development of clinically useful anticancer drugs.
- j. Kinetics of xenografts in subrenal capsule.
- k. Kinetic studies of the ovarian tumor M5076.
- l. Cell proliferation kinetics of colon tumor models.
- m. Effects of tumor passage from lungs or from subcutaneous tumors on the kinetics of mammary adenocarcinoma 16/C.
- n. Data analysis for use of cell kill as a screening endpoint.
- o. Estimates of tumor cell doubling times among male CDF1 mice from seven suppliers following intraperitoneal implant with L1210 or P388 cells.
- p. A review of recent experience with Thymidine infusion of various mouse strains bearing conventional mouse tumors and human tumor xenografts.
- q. Caloric restriction analysis of mice bearing CD8F1 mammary adenocarcinoma (first-generation transplant).
- r. Variable responses of solid tumors of mice to short duration chemotherapy: an analysis of possible causes.
- s. Absence of delayed lethality of mice treated with Aclacinomycin A.

2. Mason Research Institute (0-7325)

The purpose of this contract is to develop new in vivo tumor models with predictive value in selecting clinically effective drugs, to establish and maintain in serial transplantation human tumor cell lines in nude mice as assay systems, to validate human tumor xenografts as screening models, to develop other in vivo tumor models as indicated by Program needs, to maintain the assays developed, and to conduct special non-routine testing in assays other than the tumor panel upon specific request. The subrenal capsule (SRC) assay was developed and validated under this contract.

Over the past year, MRI has established that fresh surgical specimens can be grown and drug response evaluated in mice. Moreover, immunocompetent mice may be used. It has been feasible to predict clinical responses using a six-day SRC assay.

Retrospectively, of 20 evaluable patients, the assay predicted 13/15 positive clinical responses and 5/5 clinical failures. Prospectively, of ten evaluable patients treated on the basis of responsiveness of their tumors in the SRC assay, five responded (3 PR and 2 CR) clinically. These prospective studies are continuing in collaboration with clinical oncologists from the University of Massachusetts Medical Center and other local (Worcester area) hospitals.

With the recent implementation of our human tumor cell cloning assay (HTSCCA) projects, we have encouraged active collaboration between MRI and the Cancer Therapy and Research Foundation of South Texas (D. Von Hoff) to compare drug activity against human tumors in the HTSCCA and SRC xenograft. During this reporting period, 23 tumors (fresh surgical explants) were received at MRI from Texas. Of these, 21 grew as xenografts and were able to be evaluated in vivo. Comparison of drug effects in the HTSCCA and SRC xenograft are continuing, but the number of comparisons is too small to reach firm conclusions. It should be noted that the tumors sent by D. Von Hoff were from patients whose treatment is based on Von Hoff's findings. They are not the previously untreated patients required for our HTSCCA screens. The reason for encouraging this cooperation was to establish the practicality of such comparisons. We will propose during the next year, as a new program initiative, a contract based feasibility study to determine if human tumors transplanted serially as xenografts can provide a reproducible and stable battery of tumors of major types for in vitro testing. The subsequent in vivo testing of in vitro actives will provide data on antitumor selectivity of action; and importantly, will eliminate the need to use mice to test inactives. The work done under this contract is providing much needed background information.

In further validating the SRC model as a tumor panel screen, MRI has concluded studies which show the following:

- a. Most human tumors carried in serial transplantation in vivo have maintained a drug response profile similar to that found in the first xenograft generation.
- b. When human tumors of known drug sensitivity and tumors known to be unresponsive to the same drug were implanted bilaterally under the renal capsule of mice, the sensitive tumors responded to appropriate drug treatment while the refractory tumors did not. Similarly, when the M5076 mouse ovarian carcinoma was implanted under one renal capsule and sublines developed for resistance to Cisplatin, Hexamethylmelamine, or Melphalan were implanted under the other renal capsule, and the mice treated with the appropriate drug, the parent tumor line responded and the resistant tumor subline showed characteristic resistance. These studies were undertaken because when the change from the SC xenograft assay to the

SRC assay for tumor panel screening was proposed, there was some concern that the screening results might be due to some artifact of xenografting. These studies show clearly that the inhibition of the sensitive tumor was an expression of antitumor specificity of action and not due to the tumor implant site or mediated via host toxicity.

Mason Research has continued to characterize selected animal tumor models of interest to DEB. Emphasis was placed on the M5076 ovarian carcinoma, a model which we had previously recommended for inclusion in the tumor panel.

Mason Research continued studies related to the problem of reproducibility of screening results with the CD8F₁ mammary carcinoma tumor panel model. For screening, the first transplant generation from the spontaneous tumor is used. Inasmuch as the growth rates of the spontaneous tumors vary and this variability is reflected in the first transplant generation, the question of the characteristics of the pooled breis for inoculation of mice for screening arose. The studies showed that if one pools spontaneous tumors with dissimilar growth potentials, the resulting tumors exhibit the growth characteristics of the fastest growing individual tumor included in the pool. The results suggest that a more valid approach to drug evaluation against the CD8F₁ tumor would entail screening against a battery of individual first generation transplants.

Inasmuch as MRI maintains the frozen bank of human mammary tumors for the Breast Cancer Task Force and the frozen bank of Human and Animal Tumors for DCT, a wide variety of non-routine tumors are available for special testing. We have continued to use this contract for testing of agents purported to have hormonal activity because none of our tumor panel models is hormone responsive or dependent.

We intend to continue to use this contract for the development of new assays for drug evaluation, to provide information relative to the improvement of existing models, and for the testing of selected agents whose prior knowledge suggests the use of non-routine models not available under other contracts. A major effort will be made to augment collaborative studies between this contract and HTSCCA contractors aimed at the questions of correlations of in vitro and in vivo (SRC) results and eventual correlations with clinical efficacy.

3. Arthur D. Little, Inc. (0-7302)

This project is devoted to the evaluation of analogs, drug combinations, drugs plus radiation, and special problems arising during development. The assays (in vivo) used for analog screening are specific for each structural class and results are transmitted directly to compound suppliers to aid in further syntheses. Selected active analogs are evaluated further

against a spectrum of animal tumors, and promising analogs are recommended to NCI for further development. During the past year the contractor has designed studies to answer questions arising at various drug evaluation committee meetings at NCI (Decision Network, Radiation Sensitizer/Protector, Biochemical Modulation and Operating Committee). For example, they have provided data on the effectiveness of Thio-D-glucose as a radiosensitizer, studied the combination of Pentamethylmelamine and Pyridoxine, and described the toxicities of formulated products, such as Valinomycin.

4. Arthur D. Little, Inc. (8-7186)

The objective of this contract is to provide clear leads as to the mechanism of action of new antitumor agents. The information is used to aid DCT in assigning priorities to those drugs which are under development. To achieve this goal, ADL has followed a series of cell culture and biochemical protocols by which a biochemical profile on a new agent can be described rapidly. Some of the significant findings obtained during the past year are summarized below.

Rapamycin (NSC-226080), a structurally complex and unique antibiotic, was found to be a reasonably potent growth inhibitor of P388 cells in vitro (IC_{50} for 48 hr. exposure = $1.8\mu M$). At a growth inhibitory concentration, Rapamycin did not affect the mitotic index of P388 cells, and thus would not appear to act as a mitotic spindle poison. The drug strongly inhibited the incorporation of Thymidine into DNA at $10\mu M$, although inhibition was not evident until 30 minutes after drug exposure. At a $100\mu M$ concentration of Rapamycin, there was complete and immediate inhibition of DNA synthesis and modest inhibition (<50%) of RNA and protein synthesis. Rapamycin did not enhance the thermal stability of DNA in vitro, suggesting that the drug does not act as a DNA intercalator. However, preliminary studies using the DNA alkaline elution assay indicated that Rapamycin does react with DNA producing protein-associated DNA-strand breaks.

A second antibiotic, Aphidicolin (NSC-234714), which is active against DNA viruses, but not against RNA viruses, was shown to be a potent growth inhibitor of P388 cells in vitro (IC_{50} for 48 hr. exposure was $0.038\mu M$) and a potent selective inhibitor of Thymidine incorporation into DNA. Deoxynucleosides did not reverse the growth inhibition produced by Aphidicolin which supports the published reports that the antibiotic inhibits DNA polymerase rather than other steps of nucleic acid synthesis. The drug produced a pattern of cytotoxicity characteristic of an S-phase specific antimetabolite with long exposure times being necessary for significant levels of cell kill. However, unlike other S-phase specific agents which are more active on

an infusion schedule in vivo, Aphidicolin given at 3 hr. intervals on days 1, 5, and 9 to mice bearing P388 leukemia or B16 melanoma was no more effective than when administered as a single dose on the same days.

Structural features of a novel, synthetic antitumor agent, NSC-305884D, suggested that the compound might be a DNA intercalator. This hypothesis was substantiated by the modest effect demonstrated by NSC-305884D on the thermal denaturation of DNA. Also, initial studies indicated that sublines of P388 leukemia which were resistant to other DNA binders (Adriamycin, m-AMSA, Ellipticine) were cross-resistant to NSC-305884D. Proteinase treatment of drug treated DNA enhanced the rate of DNA elution as measured by the alkaline elution assay, indicating that NSC-305884D produces protein-associated DNA strand breaks.

This contract will expire near the end of FY 1981 and will be recompeted. Under the new contract, the biologically significant properties of new antitumor agents will be evaluated as they become of interest to DCT.

5. Institute of Cancer Research, England (4-3736)

The main tasks of this project are:

- a. Validation of human tumor xenografts as models for cancer chemotherapy.
- b. The use of xenografts and mouse tumors not used elsewhere in this Program to test new compounds for antitumor activity. The compounds tested are (a) synthesized at the Institute, (b) obtained from other sources in Great Britain or Europe (through the EORTC), or (c) identified from studies of anti-tumor drug metabolism under this contract.
- c. Toxicology required for approval for investigative clinical trial from the Committee on Safety of Medicines, animal and human pharmacology, and initial clinical trials.
- d. The design, synthesis, and testing of new drugs on the basis of the biochemical findings related to treatment response or failure.

In the past year attention has been focused on (a) the metabolism of melamines, (b) development of novel antifols, and (c) development of platinum analogs.

A comparative pharmacokinetic study of Pentamethylmelamine (PMM) in the rat, mouse and man revealed marked species differences in the oxidative N-demethylation of this agent. In several strains of mouse PMM metabolism is very rapid, leading to the production of high levels of cytotoxic N-methylolmelamines in the plasma. In the rat, however, N-methylolmelamines are correspondingly lower. Of more importance has been the finding that in patients

given an intravenous infusion of PMM, metabolism is slower still and N-methylolmelamines are not detectable. The lack of clinical activity of PMM apparent in a number of Phase I clinical studies may be due, at least in part, to the inability of patients to produce cytotoxic levels of N-methylolmelamines because of a limited capacity for oxidative N-demethylation. In order to overcome the requirement for metabolic activation and thereby achieve antitumor effects in man, the contractor has investigated the possibility of the direct administration of an N-methylolmelamine, and has shown that by giving N²,N⁴,N⁶-trimethyl,N²,N⁴,N⁶-trimethylolmelamine (CB10-375; NSC-283162) to rats it is possible to achieve peak plasma level of N-methylolmelamines comparable to those produced by PMM metabolism in the mouse. The contractor reported that NSC-283162 can be stabilized sufficiently to allow administration in an aqueous vehicle, and is no more toxic to mice than PMM. The clinical utilization of an N-methylolmelamine as an alternative to PMM may, therefore, represent a significant therapeutic advantage.

An N¹⁰-propargyl substituted quinazoline analog of folic acid (CB-3717) was synthesized and tested. This compound (no NSC number) shows substantially improved activity compared with Methotrexate against the L1210 leukemia, TLX/5 lymphoma, ADJ/PC6 plasma cell tumor and a Methotrexate refractory chorio-carcinoma grown in immune deprived mice. It is also active against MTX resistant L1210 cells. Three patents for CB-3717 have been filed on behalf of the Institute of Cancer Research and the contractor has obtained support from ICI Pharmaceuticals for its large-scale synthesis. Pre-clinical toxicology has been completed and filing of a request to the Committee on Safety of Medicines for permission to conduct a Phase I trial is planned within a month. If permission is granted, clinical studies are scheduled in the Royal Marsden Hospital for mid-summer 1981.

The contractor identified cis-diammine-1,1-cyclobutane dicarboxylate platinum II (CBDCA: NSC-241240) as the most selective Pt analog tested in this project and noted that studies at NCI and Bristol-Myers indicated that it is less emetic in the dog than is Cisplatin. The contractor has received permission from the Committee on Safety of Medicines to conduct a Phase I study on CBDCA at the Royal Marsden Hospital. Clinical studies and associated pharmacology will commence by summer 1981.

VII. Support Services

The contract with VSE Corporation (0-7251), monitored by the Automated Information Section (report appended) provided for computer processing, storage, and retrieval of data generated in testing laboratories under contract to DEB. The biological data base was used for recommending new agents for clinical trial, directing the fractionation of natural products, and directing compound acquisition and synthesis. In addition, the data base was used to provide summary information for evaluation of existing and new test systems and management of contractor ac-

tivities. A major effort was placed on analysis, design, and implementation of programs for data handling and management of human tumor stem cell cloning assays.

Although input to the screen has decreased over recent years, the complexity and amount of paperwork required to follow compounds and for data analysis has increased with the introduction of the tumor panel approach. The Screening Services contract with IIT Research Institute (9-7213) functions to: (1) assist in maintaining the flow of materials to contract screening laboratories; (2) maintain the files on selected agents; (3) review primary xenograft screening data for presumptive activity and schedule the compounds for additional testing as required; (4) prepare letters to suppliers to notify them of the status of their compounds; (5) prepare the data for the Pre-screen and Data Review Subcommittee meetings and the minutes of these meetings; (6) schedule compounds for testing in the panel of tumor test systems as recommended by staff; (7) review and summarize test results as requested; and (8) provide data processing support for assistance in management of the screening program. In addition the contractor maintains a computerized Transaction File on compounds of interest to DTP, maintains and distributes the Operating Committee File, another computerized system which includes the minutes of the Committee meetings and all important data on compounds being followed by the Decision Network.

VIII. Future Course

DEB will continue to manage its world-wide program for the recognition and development of new and improved anticancer therapies with maximal accountability. We will pursue new initiatives as indicated by fundamental studies and our judgment of prognosis for success. We will continue to survey grant funded research projects for indications of methodological improvements, but must continue to utilize the contract mechanism to develop methods aimed specifically at our targeted program. The diversity of activities managed by DEB is a reflection of the premise that grants are given to support research and contracts are written to procure research; the difference being related primarily to immediate need for information rather than the nature of the proposed work. DEB objectives are staff initiated and directed to the fulfillment of DCT objectives. Approaches to realization of objectives may be staff initiated, contractor initiated, or the result of active collaborations. DEB will continue to devote its best effort in improving resources to carry out the overall DCT drug development program and to offer its best advice and counsel to Program on any matter that falls within the competence and experience of its staff.

TABLE 1

DEB CONTRACTORS AND FUNDING LEVELS,* FY 1981

1. <u>In Vivo Screening</u>	<u>\$ 4,525,614</u>
Arthur D. Little, Inc. (0-7346)	525,603
Battelle Memorial Institute (0-7266)	940,825
Battelle Memorial Institute (0-7099)	520,930
IIT Research Institute (9-7316)	973,378
Institute Jules Bordet, Brussels (0-7350)	199,286
Mason Research Institute (9-7317)	1,365,592
2. <u>In Vitro Screening</u>	<u>\$ 1,250,405</u>
Arthur D. Little, Inc. (9-7288)	94,534
University of Miami (9-7290)	174,180
University of Arizona (1-7497)	187,850
Mayo Clinic (0-7419)	205,049
University of California at Los Angeles (0-7420)	241,500
Cancer Therapy and Research Foundation of South Texas (0-7327)	347,292
3. <u>Detailed Drug Evaluation</u>	<u>\$ 2,645,693</u>
Arthur D. Little, Inc. (8-7186) (RFP-TI-7397)	150,000
Arthur D. Little, Inc. (0-7302)	487,456
Southern Research Institute (9-7309)	2,008,237
4. <u>International Drug Development</u>	<u>\$ 105,000</u>
Institute of Cancer Research, England (4-3736)	105,000
5. <u>Model Development</u>	<u>\$ 202,922</u>
Mason Research Institute (0-7325)	202,922
6. <u>Data Processing and Support Services</u>	<u>\$ 1,286,997</u>
VSE Corporation (0-7251)	987,547
IIT Research Institute (9-7213)	299,450
TOTAL	<u>\$10,016,631</u>

*Negotiated or estimated.

TABLE 2

DEB STAFF PARTICIPATION ON DCT DECISION GROUPS, FY 1981

Cancer Treatment Program Staff
DCT Decision Network Committee
DCT Operating Committee
DTP Pre-Decision Network Committee
Developmental Therapeutics Resource Contract Review Committee*
DTP Senior Staff Contract Review Group
DTP Analog Development Committee
DTP Steering Group for Computer Processing*
DCT Radiosensitizer/Radioprotector Analog Committee
DEB Drug Evaluation Committee*
DEB Data Review Subcommittee*
DEB Pre-Screen Subcommittee*
Cancer Treatment Reports, Editorial Board

*Chaired by DEB senior staff member.

TABLE 3

TESTING STATUS OF TUMOR PANEL (TP) COMPOUNDS (MARCH 23, 1981)

Total number of compounds entered into TP	1,817
Number dropped prior to completion of testing	105
Number of TP compounds excluding "drops"	1,712
Total number completed in all TP test systems	671
Number completed between April 1, 1980 and March 23, 1981	406

TABLE 4

TESTING STATUS BY TEST SYSTEM FOR 1,712 TUMOR PANEL COMPOUNDS
(MARCH 23, 1981)

Test System	Completed Testing	On Test or Awaiting Testing	Not Available*
		Number (%)	
<u>Mouse Tumors</u>			
Bl6 melanoma	1,370 (80)	119 (7)	223 (13)
CD8F ₁ mammary carcinoma	1,322 (77)	111 (7)	279 (16)
Colon 38	1,357 (79)	110 (7)	245 (14)
Leukemia L1210	1,384 (81)	102 (6)	226 (13)
Lewis lung carcinoma	1,358 (79)	116(7)	238 (14)
<u>Human Tumor Xenografts (Renal Capsule Assay)⁺</u>			
CX-1, Colon	997 (58)	393 (23)	322 (19)
LX-1, Lung	930 (55)	468 (27)	314 (18)
MX-1, Mammary	793 (46)	600 (35)	319 (19)

*QNS (Quantity not sufficient to complete testing).

+Includes approximately 80 compounds evaluated against subcutaneous xenografts prior to institution of the renal capsule assay.

TABLE 5

TUMOR PANEL COMPLETION RATE FOR COMPOUNDS AVAILABLE FOR TESTING
(MARCH 23, 1981)

Test System	Number Completed Completed, On Test, And Scheduled	Percentage Completed
<u>Mouse Tumors</u>		
Bl6	1370/1489	92
CD8F ₁	1322/1433	92
Colon 38	1357/1467	93
L1210	1384/1486	93
Lewis lung	1358/1474	92
<u>Xenografts</u>		
CX-1, Colon	997/1390	72
LX-1, Lung	930/1398	67
MX-1, Mammary	793/1393	57

TABLE 6

CUMULATIVE COMPLETION RATE FOR INDIVIDUAL TUMOR PANEL MODELS THROUGH
THE LAST THREE REPORTING PERIODS

	<u>Cumulative Reporting Period</u>		
	Through 3/31/79 (1,200)	Through 3/31/80 (1,487)	Through 3/23/81* (1,712)
TP Compounds (Total)			
<u>Test System</u>	<u>Number Completed (%)</u>		
<u>Mouse Tumors</u>			
B16	830 (69)	1,019 (69)	1,370 (80)
CD8F ₁	640 (53)	862 (58)	1,322 (77)
Colon 38	734 (61)	989 (67)	1,357 (79)
L1210	930 (78)	1,059 (71)	1,384 (81)
Lewis lung	666 (56)	941 (63)	1,358 (79)
<u>Human Tumor Xenografts</u>			
CX-1, Colon	94 (8)	443 (30)	997 (58)
LX-1, Lung	133 (11)	502 (34)	930 (55)
MX-1, Mammary	130 (11)	441 (30)	793 (46)

TABLE 7

NUMBER OF COMPOUNDS EVALUATED IN EACH TUMOR PANEL MODEL FROM
APRIL 1, 1980 THROUGH MARCH 23, 1981

<u>Test System</u>	<u>Number</u>	<u>Difference from Average</u>
B16	351	- 56
CD8F ₁	460	+ 53
Colon 38	368	- 39
L1210	325	- 82
Lewis lung	417	+ 10
CX-1 Xenograft	554	+147
LX-1 Xenograft	428	+ 21
MX-1 Xenograft	352	- 55*
Average of all models	407	0
Completed in all models	406	0

TABLE 8

PROJECTED TESTING NEEDS TO COMPLETE 2,000 COMPOUNDS IN ALL DCT TUMOR
PANEL SYSTEMS

Test System	Completed 3/23/81	Remaining	Years to Complete at 400/year
B16	1,370	630	1.6
CD8F ₁	1,322	678	1.7
Colon 38	1,357	643	1.6
L1210	1,384	616	1.5
Lewis lung	1,358	642	1.6
CX-1 Xenograft*	997	1,003	2.5
LX-1 Xenograft*	930	1,070	2.7
MX-1 Xenograft*	793	1,207	3.0

TABLE 9

RELATIVE COST PER TEST* USING TUMOR PANEL MODELS

Test System	Relative Cost
L1210 ⁺	1.0
B16	2.0
Lewis lung	2.75
CD8F ₁	3.0
Colon 38	3.0
CX-1 Xenograft, Renal Capsule	6.0
LX-1 Xenograft, Renal Capsule	6.0
MX-1 Xenograft, Renal Capsule	6.0
CX-1 Xenograft, Subcutaneous ⁷	10
LX-1 Xenograft, Subcutaneous ⁷	10
MX-1 Xenograft, Subcutaneous ⁷	10

*A test is defined as one experimental group of 6-10 treated animals.

⁺All costs are expressed as L1210 equivalents with the cost of an L1210 test defined as 1.0.

⁷No longer used.

TABLE 10

**YIELD OF COMPOUNDS MEETING DN2 ACTIVITY CRITERIA
AGAINST TUMOR PANEL MODELS***

Tumor	% DN2	% MC-1	% Inactive
Prescreen			
P388	28.4	61.3	10.3
Tumor Panel			
L1210	22.1	21.4	56.5
Melanoma	18.2	17.2	64.6
Mammary			
Human	12.6	1.5	85.9
Murine	9.5	21.1	69.4
Lung			
Human	4.9	2.7	92.4
Murine	2.9	3.4	93.7
Colon			
Human	2.7	1.0	96.3
Murine	2.5	15.5	82.0

*Based on 588 compounds with testing complete in all panel systems.

TABLE 11

**IS THE TUMOR PANEL SELECTING AGENTS FOR
CLINICAL TRIAL THAT WOULD HAVE BEEN MISSED BY
SCREENING WITH L1210 LEUKEMIA ALONE?**

Number of compounds with less than DN2 activity against L1210^a = 458

Number with DN2 level activity in some other tumor panel model = 103

Murine Tumor	Number of DN2 Actives	Human Tumor Xenograft	Number of DN2 Actives
Melanoma	68		
Mammary	19	Mammary	36
Lung	4	Lung	13
Colon	4	Colon	6

^a458 of 588 compounds with testing complete in all tumor panel models.

TABLE 12

**ARE THE HUMAN TUMOR XENOGRAPTS AND MURINE TUMORS
SELECTING THE SAME OR DIFFERENT DRUGS?**

Number of compounds with DN2 activity in at least one of the xenograft models = 89

Number not demonstrating DN2 activity in the murine tumor panel models = 18

Xenograft	Number of DN2 Actives In Xenografts Only		Examples of Pre- Clinical Compounds
	Clinical	Pre-Clinical	
Mammary only	4	6	Alkylating Agents, DMF, purine analog
Lung only		4	Triazenes, acridine derivative
Colon only		1	Pep-bleomycin
Mammary + Lung	1		
Mammary + Lung + Colon		2	N-methylformamide Mithramycin analog

TABLE 13

**ARE THE HUMAN TUMOR XENOGRAPTS AND THE
MURINE TUMORS OF A SPECIFIC ORGAN SYSTEM
SELECTING THE SAME DRUGS?**

Organ	Number of Compounds Exhibiting DN2 Activity			
	Human Tumor Only	Murine Tumor Only	Both	Neither
Colon	13	11	2 ^a	491
Lung	24	10	4 ^b	483
Mammary	47	26	24 ^c	420

^aAraC and vinblastine analogs.

^bNitrosoureas.

^cMainly alkylating agents and DNA intercalators/binders.

TABLE 14

PRE-SCREENS FOR NATURAL PRODUCTS
(Example of Enrichment Process)

1979 Fermentation Statistics			
<u>Cultures</u> <u>Fermented</u>	<u>Active</u> <u><i>In Vitro</i></u>	<u>Tested</u> <u><i>In Vivo</i></u>	<u>Active</u> <u><i>In Vivo</i> (P388)</u>
15,317	3,595 (23%)	1,161	180 (16%)

TABLE 15

PRE-SCREENS FOR NATURAL PRODUCTS

New Leads: Search for compounds using a variety of biological and biochemical assays

Examples (under development):

1. Astrocytoma assay
2. *Candida albicans*
3. *Xanthomonas*
4. *Agrobacterium*
5. Aminopeptidase B
6. Beta galactosidase phage induction

FIGURE 1

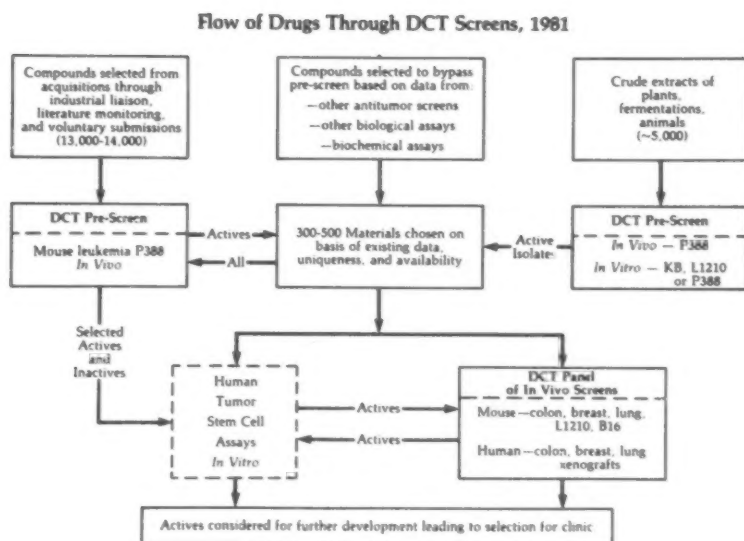


FIGURE 2

CURRENT DCT IN VIVO SCREENING SYSTEMS

PRESCREEN

P388 lymphocytic leukemia

TUMOR PANEL

I. Murine tumors

B16 melanocarcinoma
L1210 lymphoid leukemia
Colon 38 carcinoma
Lewis lung carcinoma
CD8F₁ mammary carcinoma

II. Human tumor xenografts

CX-1 colon tumor
LX-1 lung tumor
MX-1 mammary tumor

FIGURE 3

TECHNIQUE FOR TUMOR STEM CELL ASSAY

Prepare single cell suspension (from ascites, pleural fluid or solid tumor).

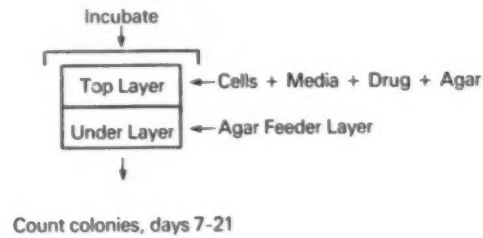


FIGURE 4

DESIGN OF PILOT STUDY

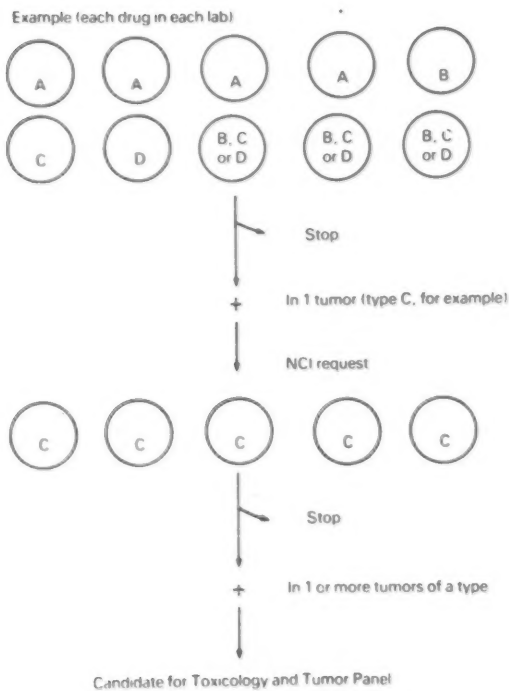


FIGURE 5

PILOT PHASE

KEY QUESTIONS

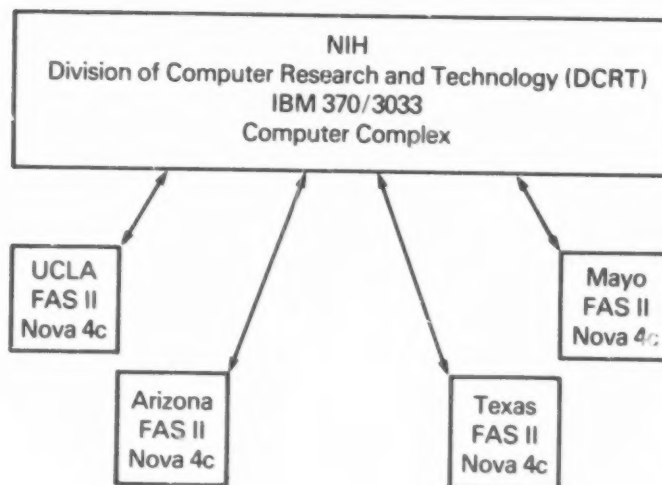
1. What is the response of each of 50 "blinded" compounds against a minimum of 40 different human tumor samples?
2. What are appropriate activity criteria?
3. What is an appropriate tumor mix?

DEVELOPMENTAL STUDIES FOR SCREEN

1. Can one or more media be selected?
2. How do enzyme and mechanical disaggregation techniques compare?
3. Can frozen specimens be used?

FIGURE 6

**STEM CELL DATA COMMUNICATIONS
(On-Line Conversational System)**



Publications by Staff

1. Venditti, John M.: Pre-clinical drug development: Rationale and methods. Seminars in Oncology (in press).
2. Venditti, John M.: The Model's Dilemma (in press).
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3. Brockman, R. W.: Resistance to therapeutic agents. In Burchenal, J. H. and Oettgen, H. F. (Eds.): Cancer Achievements and Challenges for the 1980's 2. New York, Grune & Stratton, 1981, pp. 55-66.
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9. Griswold, D. P., Corbett, T. H., and Schabel, F. M.: Pharmacology and development of new therapeutic agents. Gastrointest. Cancer (in press).
10. Griswold, D. P., Schabel, F. M., Corbett, T. H., and Dykes, D. J.: Concepts for controlling drug-resistant tumor cells. Design of Models for Screening of Therapeutic Agents for Cancer (in press).
11. Johnson, R. K., Howard, W. S., Faucette, L. F., Wodinsky, I., and Clement, J. J.: Influence of Pyrazofurin on the toxicity and anti-tumor activity of fluorinated Pyrimidines in vivo. Advances in Enzyme Regulations (in press).
12. Ovejera, A. A. and Houchens, D. P.: Human tumor xenografts in athymic mice as a pre-clinical screen for anticancer agents. Semin. in Oncol. (in press).
13. Schabel, F. M.: Laboratory methods for the detection and development of clinically useful anticancer drugs. Cancer: Achievements, Challenges and Prospects of the 1980's (in press).
14. Schabel, F. M., Laster, W. R., Trader, M. W., Corbett, T. H., and Griswold, D. P.: Combination chemotherapy with Nitrosoureas plus other anticancer drugs against animal tumors. Nitrosoureas: Current Status and New Developments (in press).
15. Schabel, F. M., Griswold, D. P., Corbett, T. H., Laster, W. R., Lloyd, H. H., and Rose, W. C.: Variable responses of advanced solid tumors of mice to treatment with anticancer drugs. Design of Models for Screening of Therapeutic Agents for Cancer (in press).
16. Schabel, F. M., Skipper, H. E., Trader, M. W., Laster, W. R., Corbett, T. H., and Griswold, D. P.: Concepts for controlling drug-resistant tumor cells. In Mourisden H. I. and Palshof, T. (Eds.): Breast Cancer: Experimental and Clinical Aspects. Oxford, England, Pergamon Press, 1980, pp. 199-211.
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AUTOMATED INFORMATION SECTION

DRUG EVALUATION BRANCH

The Automated Information Section (AIS) is responsible for the implementation and maintenance of the biological data processing system for the Drug Evaluation Branch (DEB), thereby providing for the automated processing, storage, and retrieval of biological data generated in testing laboratories under contract to DEB, Developmental Therapeutics Program (DTP). Synthetic and natural products are tested in animals for antitumor activity and in cell cultures at various screening laboratories prior to data entry into the system. On the basis of these test results, decisions are made concerning the direction of compound acquisition, synthesis, fractionation of active crude natural products, and the recommendation of new agents for development toward clinical trial. The biological data base is capable of being queried by special programs thus providing special summary data for: (1) evaluation of the existing screens; (2) design and evaluation of new test systems; (3) review of antitumor activity by the Drug Evaluation Committee, Operating Committee, and Decision Network Committee; (4) detailed evaluation of individual compounds and drug combinations; (5) reports to be included in Investigational New Drug Applications and their updates; and (6) statistical information requested for effective Program management.

The AIS is staffed by two professionals, and two junior programmers. Professional staff serves as project officer and has one contract with VSE Corporation (N01-CM-07251). This contract has been supplemented twice. The first supplement was for the analysis, design, and implementation of programs for processing the data generated in the Human Tumor Stem Cell Cloning (HTSCC) Assay. The second supplement was for producing 16 mm microfilm from computer output microfilm files.

During this period the Synthetic and Selected Agent Master File was split into three files. A permanent archive file, is one on which data from negative and inactive agents are stored. An archive file, where data meeting different criteria is of more recent testing but also negative and inactive reside. The current active file, where data which are active and have resulted from testing in a recent time frame resides. All three files are searchable by equivalent criteria simply by specifying which of the files is to be searched. The master files (Synthetic/Selected Agent Compound and Natural Product) were microfilmed as well as the Plant Header Master File. Major corrections to the Plant Header Master File were made including corrections to the location of collection codes necessitated by renaming of some of the Third World Countries. In addition to these corrections major corrections to the taxonomy of the collected specimens were made by means of a file of accepted taxonomic names obtained from the Smithsonian Institution.

The AIS also provided various reports for the Screening Section (S.S.), DEB including analogue/parent comparison of data for the Anthracycline Analog Committee and statistical reports analyzing in vitro data and solid tumor data. Again, during this report period, the Drug Synthesis and Chemistry Branch was provided a data file for the ongoing Chemical Information System pattern recognition project.

There is a standing "Quantity Not Sufficient" (QNS) query run biweekly for the IITRI Screening Services contract (N01-CM9-7213) to aid in the scheduling of retests of active compounds. The QNS report lists those materials which need to be acquired and informs staff when they are available. Prior to every Operating Committee meeting a special query and report is run for the Screening Section. Manually generated data extracted from these reports are then input to the Operating Committee Report System and/or the Compounds of Interest File.

The Natural Products Branch (NPB), again received data processing support including the monthly Materials of Interest Report and the quarterly P388/ L1210 Report. In addition, several large queries were also run for the NPB in order that material selection methods might be refined and that contractor activity might be more closely monitored. The NPB also requested and received special Analogue/Parent compound comparison reports for the Bleomycin and Mitomycin classes to aid them in compound selection.

During the reporting period over 225 queries were processed from in-house and out-of-house for biological data. The newly formed Pre-Screen Subcommittee of the Drug Evaluation Committee requested and received queries on almost a bi-weekly basis. This group reviews compounds with biological activity and also reviews requests for direct input to the Tumor Panel.

As in past years, commercial suppliers have requested, in machine readable form, data on their compounds. These requests were satisfied and encouraged as these suppliers provide many compounds to the Program.

The Operating Committee and Compounds of Interest Reports were continually updated during this period and distributed to all members prior to each meeting thus providing each member with up-to-date information. The Operating Committee Report is at the present time being studied with the intention of modifying it. These modifications will be finished during the next reporting period.

One of the biggest tasks undertaken this period was the analysis, design, and implementation of the HTSCC assay. This is a new in vitro screening pilot project which involves four new screeners. This project involves the use of a Bausch and Lomb FAS II Image Analysis System which has a mini-computer (NOVA 4) as its controller and processor. In order that the data, proper inventory of compounds, screening and telecommunications with the DCRT be transmitted in a timely fashion, a NOVA 4 had to be procured. The pathology of the human tumors involved required the use of the "International Classification of Diseases for Oncology (1976) be used. The Division of Cancer Cause and Prevention (NCI) provided a machine readable version which had to be tailored to fit the needs of the AIS and VSE Corporation.

Another major task required of the AIS was the analysis, design and implementation of the in vitro Astrocytoma screening system. This required that data from a new screen be input, edited, updated on the master file, and reported to both suppliers of compounds and staff for evaluation. Staff includes both DEB and NPB scientists.

The biological data processing system was expanded to include four new tumor systems and twenty new test systems.

The Solid Tumor System in use since 1977 was redesigned. This was done in such a fashion that the Solid Tumor Terminal Handler System and the input forms were not affected, but the file design, processing programs and the output reports were reprogrammed. This was accomplished in the Spring of this report period.

The SAC off-line and on-line systems were combined for the first time during this period. Thus, DEB personnel are capable of updating the SAC on-line file in a much less time consuming manner and the changes affected during a biweekly cycle are reflected on the Screening Data Summaries produced during that cycle.

It is clear from this report that AIS is a necessary and responsive resource for DEB. The continuing effort devoted to new systems development and current data processing methodologies represent our attempt to meet the needs of the Branch regardless of modifications in test systems, experimental protocols, and priorities. The increasing complexity of testing procedures and the need for rapid summarization of results from the contract testing laboratories throughout the world has progressively increased the need to use automated data processing systems. In addition, in its effort to maximize efficiency in the use of funds, testing capacity and animal resources, DEB has relied heavily on AIS to provide accurate and current utilization reports. Thus, AIS occupies a focal position in the DEB organization and plans to continue to be responsive to Program needs. A major goal for the coming year will be the continuing effort to integrate the printing of biological and chemical data on the same report.

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SCREENING SECTION

DRUG EVALUATION BRANCH

The principal objectives of the Screening Section are the evaluation and recommendation of new materials as candidates for development to clinical trial against cancer and the investigation of means for increasing the therapeutic usefulness of known agents. Objectives are attained through contractual agreements with qualified institutions for (a) pre-screening synthetic and natural products against P388 leukemia in mice, or to a limited degree in vitro, (b) screening of selected compounds against a panel of murine tumors and human tumor xenografts, (c) bioassay of natural product fractions leading to isolation of the active component, (d) treatment route and schedule dependency studies, (e) methodology studies leading to improved antitumor screens. Results are presented to Division of Cancer Treatment (DCT) staff. Activity against experimental tumors in vivo is the principal basis for recommending materials for development toward clinical trial. In vitro assays are used for synthetic materials submitted in too small a quantity for in vivo testing and for following the isolation of an active moiety of a crude natural product and correlating the cytotoxicity with in vivo activity.

I. Staff Functions

During this report period, staff served as Project Officers for six contracts for in vivo screening. Arthur D. Little, Inc. (N01-CM0-7346), Battelle-Columbus Laboratories (N01-CM0-7266 and N01-CM6-7099), IIT Research Institute (N01-CM9-7316), Institut Jules Bordet (N01-CM0-7350), and Mason Research Institute (N01-CM9-7317). The annual test capacity at these six laboratories was 205,000 L1210 equivalency tests in the P388 leukemia pre-screen and in eight tumor panel models: five mouse tumor test systems (B16 melanoma, CD8F1 mammary with carcinoma, colon 38, L1210 leukemia, and Lewis lung carcinoma; and three human xenografts, mammary (MX1), colon (CX1), and lung (LX1), in which human tumor is implanted under the kidney capsule of athymic mice. A screening services contract with IIT Research Institute (N01-CM9-7213) assisted staff in the management of screening data.

Staff also served as Project Officers for three in vitro screening contracts: Arthur D. Little, Inc. (N01-CM9-7288), University of Wisconsin (N01-CM9-7289) and the University of Miami (N01-CM9-7290). The University of Wisconsin contract terminated during this report period.

Staff collaborated with the Office of the Chief, DEB, the Natural Products Branch (NPB), and the Office of the Director, DCT in monitoring screening related tasks performed under contract to those units; and reviewed the biological effects of materials produced under additional DTP contracts for natural product production, fractionation, and isolation.

Contracts assigned to the Screening Section were closely monitored to assure that the testing adhered to the goals of the DTP, DCT, and NCI. Staff continually selects new assays for potential use in the search for new antineoplastic drugs, designs and evaluates existing and new protocols for use by the contract laboratories, evaluates test results, recommends materials for development toward clinical trial, and prepares data summaries for presentation to appropriate decision making groups.

II. Significant Accomplishments

A. Determination of Antitumor Activity or New Agents

Primary screening in vivo consists of the initial testing for activity against transplantable mouse tumors. Protocols are designed to uncover a modest number of materials which are then subjected to progressively exacting requirements to select those with the greatest potential.

Transplantable mouse leukemia P388 remained the initial in vivo screen for most materials submitted for testing. The reasons for selecting P388 as the "pre-screen" were reported previously. Materials which are active in the pre-screen, or which "by-pass" the pre-screen because of demonstrated interesting activity outside the program and some of special interest because of the rationale for synthesis or structure are tested against the panel of eight experimental tumor models. Other tumors such as brain, renal, ovarian or bladder may be used on a selective basis when appropriate. Materials regarded as potential candidates for clinical trial are examined, prior to large animal toxicology studies, in all of the tumor panel test systems as well as for route and schedule dependency. Analogs of compounds in development are compared with the biological activity of the parent. Experimental and final clinical formulations are tested for activity whenever appropriate.

Crude plant and animal products are tested in vitro against the rat astrocytoma cell line for cytotoxicity and reversal of astrocyte formation, as well as in vivo against P388 leukemia in mice. Where activity is observed in both, the less expensive cell culture assay is used for bioassay to follow activity during fractionation procedures. The KB and P388 cell lines continue to be utilized for the bioassay of materials active in these systems, and have in vivo activity also. Tables 1 and 2 show the number of screening tests initiated and computer processed during the period of April 1, 1980 through March 31, 1981. An in vivo test is defined as one treated group of from six to ten animals. An in vitro test consists of a concentration - response assay from which the cytotoxic ED₅₀ is extrapolated.

Of 161,654 total tests for this 12-month period, 146,693 were in vivo (Table 1) as compared with 171,609 in vivo of a total of 185,252 tests for the same period last year. A total of 14,961 in vitro tests (14,009 were natural product assays) were conducted

this year (Table 2). Tables 1 and 2 also list the number of tests conducted with "selected agents" (SAC) which are chosen for further study because of activity in initial screening or other biological activity of interest, other synthetic compounds (Regular), structural congeners of known active compounds (Analog), crude plant extracts, crude fermentation beers, crude animal extracts and natural product fractions. These numbers include tests processed in our main computerized single drug data file. They exclude special testing such as combination chemotherapy or testing in new systems under current development for which results are not computer processed. For example, there were about 60 new unique drug combinations tested during this same period which are not included in these tables.

During this reporting period, testing was initiated and results computer processed for 22,483 materials, both synthetic and natural products (Table 3), as compared with 21,563 during the previous year. Of the 22,483 new materials, 13,799 were synthetic agents. These counts are made from the date the materials were first tested and not the date of accession of the materials. Table 3 also shows the average lag time between the date a test is initiated and the date that test results are computer processed. This lag time is greatest for selected agents and less for other synthetics or natural products. In general, initial screening is completed in a relatively short time. As materials are determined to be active or require specialized testing, they are moved to the SAC file. The longer lag time for the latter is a reflection of activity (animal survival), more sophisticated test systems requiring more time for completion, and the greater number of test systems. This is demonstrated in Table 4. The number of tests is less in the last quarter reported, because of this lag time, and not because of a decrease in the number of tests initiated.

Table 4 shows, by test system, the number of tests carried out during this report period in individual tumor models including testing of compounds in the complete panel and testing of compounds in a limited spectrum of panel tumors, e.g., analogs of materials of special interest and antibiotics selected from in vitro or biochemical pre-screens. The M5076 ovarian carcinoma model is included in Table 4 as an example of the ancillary test system. Table 4 also shows the number of xenograft tests using the subcutaneous (H2) and subrenal capsule (G5) sites of tumor implantation. The value of the subrenal capsule site was reported last year. During the current year, routine panel testing included the subrenal capsule assay only. The subcutaneous site continues to be used by special request only. The number of xenograft tests is lower than the number of tests in the conventional mouse tumor systems. Four laboratories now have the special barrier facilities to carry out testing with the xenograft systems; however, the overall test capacity for the xenograft systems is limited. Additionally, the

special requirements for xenograft testing limit flexibility, and one laboratory was closed for two months due to a Sendai virus outbreak.

Candidate drugs for pre-clinical toxicology (DN5) are required to have testing completed in all panel systems. Because test capacity for the xenograft systems has been limited, priority in these systems continues to be given to the testing of those agents beyond Decision Network 2A in the Linear Array. During this reporting period, 460 materials were evaluated as having testing complete in the tumor panel. As of March 31, 1981, 1,828 compounds had been selected for tumor panel testing; 252 during this reporting period. These were selected by the following criteria:

200 new compounds on pre-screening activity, or known compounds for comparison testing with a new compound, selected by the Data Review and Pre-screen Subcommittees.

6 analogs selected by coordinating committees;

46 by-pass compounds selected by staff and committee.

B. Selection of Candidate Drugs for Clinical Trial

The Selected Agents (SAC) file was augmented by 649 compounds this fiscal year (Table 5). These compounds demonstrated confirmed activity in vivo (640) or in vitro (9). The majority of in vivo actives, 594, were selected initially by the P388 screen.

The Data Review Subcommittee of the Drug Evaluation Committee and the new Pre-screen Subcommittee together reviewed screening data for 1,566 materials (Table 6), referring 49 compounds, along with their analogs in many cases, to the DEC. The DEC reviewed a total of 72 compounds representing DRS referrals, various Analog Committee referrals, as well as non-Program referrals. Table 7 lists all agents selected for further development during this reporting period, by the Decision Network group and indicates the basis for their selection. It includes those compounds recommended at decision point 2A as well as those which entered the linear array at decision points beyond 2A.

C. Detailed Evaluation of New and Old Drugs of Particular Interest

The Screening Section is responsible for the detailed evaluation of drugs which pass DN-2A. Results are reviewed and summarized in collaboration with the office of the Chief, DEB, for Decision Network presentations subsequent to DN-2A, for inclusion in clinical brochures and IND applications, and for presentation to clinical working groups.

Fourteen treatment route and schedule dependency studies were initiated this year on materials in development towards clinical trials.

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Fourteen experiments comparing antitumor activity bulk drugs and experimental clinical formulations were conducted on agents in development toward clinical trial. In some cases, the evaluation of a number of experimental forms for an individual drug was required to insure against loss or significant reduction of antitumor efficacy during the formulation process.

In vivo testing of congeners of clinically active antitumor drugs was conducted at Arthur D. Little, Inc. (NO1-CM5-3765) using animal-tumor models selected on the basis of established antitumor activity of the parent compound. Congeners with promising antitumor activity relative to the parent drug, are selected for Program development. The project also provides useful information in a timely manner, to medicinal chemists in this and other drug development programs. The project is designed to: a) identify drug classes of special interest, in particular the major classes of clinically active drugs; b) acquire new structural congeners as well as other rationally-designed drugs; and c) compare congeners with parents to determine their potential superiority or uniqueness.

D. Methodology (Research and Development)

During this past year, evaluations of the protocol changes noted in the last Annual Report -- CD8F1 murine mammary tumor and the xenograft tumor models -- as well as the substitutions of B6C3F1 hybrid mouse for the BDF1 hybrid mouse as test host for the Lewis lung tumor system were monitored.

A workshop was held to discuss the effect of caloric restriction and related weight loss on the growth of the CD8F1 mammary tumor. It was deemed essential to review this data as many materials displaying efficacy in this system cause considerable weight loss at efficacious doses. An analysis of weight loss effect on tumor size revealed that this tumor is not sensitive to weight loss of the host animal.

The three (3) subrenal capsule xenograft protocols having been finalized, a statistical analysis of the reproducibility within and between laboratories was initiated, with the assistance of the Biometric Research Branch, DCT. For this study, the MX-1 (3MBG5) xenograft model was selected because a sufficient number of materials had been tested in this system to permit the selection of compounds that were active as well as inactive. Sixteen materials were selected and sent to three contract laboratories (Battelle Columbus, IITRI, Mason Research Institute) to be tested under "blind" NSC numbers. An evaluation of the preliminary results of this study indicates that there is reproducibility within and between laboratories.

During this report period, the rat astrocytoma in vitro protocol was finalized and all crude natural product extracts are scheduled

for testing in this system in addition to the in vivo testing. The 9KB, P388 and L1210 in vitro systems are utilized only for the confirmation of previously presumptive cytotoxic materials and/or the following of the purification or previously confirmed cytotoxic natural products.

III. Related Projects

The Screening Section, with the close cooperation of the Animal Genetics and Production Branch, maintains strict surveillance of all tumor lines and host animals to assure the uniformity of biological characteristics. Frozen tumor banks are maintained at several in vivo and in vitro screening laboratories as well as a major frozen tumor bank under contract to the Animal Genetics and Production Branch (AG&PB). All studies related to the diagnosis and control of animal disease, and general animal quality are supervised by AG&PB. The AG&PB advises the Screening Section of anticipated animal shortages or problems and remedies -- such as, changes of host animals -- which are made.

The Screening Section is the major user of the biological automated data processing system and collaborated closely with the Automated Information Section, DEB, to institute new programs or modify existing programs.

IV. Proposed Course of Projects

The Screening Section will continue to supervise the testing and evaluation of materials screened in the DTP Program. This includes the continuous evaluation of the primary screen and its criteria, logistics, and tumor models. Research and methodology will be carried out at contract laboratories, directed towards the improvement of experimental tumor models and the evaluation of new models for potential usefulness as screens. Finalized protocols for use by all contract laboratories will be prepared.

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TABLE 1

NUMBER OF IN VIVO SCREENING TESTS CONDUCTED FROM
APRIL 1, 1980, THROUGH MARCH 31, 1981*

	SELECTED AGENTS COMPOUNDS (SAC)		OTHER SYNTHETICS		NATURAL PRODUCTS			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentation	Total
<u>1980</u>								
Second Quarter	14,539	1,110	10,901	367	7,030	643	3,905	11,578
Third Quarter	13,349	1,106	10,198	469	7,856	1,962	4,222	14,040
Fourth Quarter	9,343	1,049	11,842	734	9,772	2,504	3,441	15,717
<u>1981</u>								
First Quarter	6,567	451	11,474	634	7,960	1,273	1,932	11,165
TOTAL	43,798	3,716	44,415	2,264	32,618	6,382	13,500	52,500

	Second Quarter 1980	Third Quarter 1980	Fourth Quarter 1980	First Quarter 1981	Four- Quarter Period
TOTAL ALL IN VIVO TESTS	38,495	39,162	38,685	30,351	146,693

*Includes tests begun no earlier than April 1, 1980, and reported to the Automated Data Processing (ADP) Contractor by April 10, 1981.

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TABLE 2

NUMBER OF IN VITRO SCREENING TESTS CONDUCTED FROM
APRIL 1, 1980, THROUGH MARCH 31, 1981*

	SELECTED AGENTS COMPOUNDS (SAC)		OTHER SYNTHETICS		NATURAL PRODUCTS			
	Regular	Analog	Regular	Analog	Plants	Animals	Fermentations	Total
<u>1980</u>								
Second Quarter	42		26		2,524	695	7	3,226
Third Quarter	141		50		2,605	460	30	3,095
Fourth Quarter	394		28		3,027	717	172	3,916
<u>1981</u>								
First Quarter	241		30		2,662	406	704	3,772
TOTAL	818		134		10,818	2,278	913	14,009

	Second Quarter 1980	Third Quarter 1980	Fourth Quarter 1980	First Quarter 1981	Four- Quarter Period
TOTAL ALL IN VITRO TESTS	3,294	3,286	4,338	4,043	14,961

*Includes tests begun no earlier than April 1, 1980, and reported to the Automated Data Processing (ADP) Contractor by April 10, 1981.

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TABLE 3

SUMMARY OF MATERIALS FIRST TESTED IN ANY SYSTEM FROM
APRIL 1, 1980, THROUGH MARCH 31, 1981*

	SELECTED AGENTS COMPOUNDS (SAC)			SYNTHETICS			NATURAL PRODUCTS				TOTAL ALL
	Synthetic	Analog	Total	Synthetic	Analog	Total	Plants	Animals	Fermen- tations	Total	
1980											
Second Quarter	987	118	1,105	3,217	68	3,285	1,756	206	345	2,307	6,697
Third Quarter	355	46	401	2,583	82	2,665	1,191	407	288	1,886	4,952
Fourth Quarter	246	30	276	2,957	120	3,077	1,656	411	377	2,444	5,797
1981											
First Quarter	83	6	89	2,825	76	2,901	1,473	174	400	2,047	5,037
TOTAL	1,671	200	1,871	11,582	346	11,928	6,076	1,198	1,410	8,684	22,483

Analysis of lag time between the date the tests were initiated at the laboratory (date-on) and the date the test results are processed reveals that the following percentages of the data have been processed in the first, second, and third months beyond the date-on month:

Month Beyond Date-On	SAC		Synthetics		Natural Products	
	Average	Range	Average	Range	Average	Range
First	45%	28% - 63%	52%	27% - 71%	66%	49% - 85%
Second	82%	67% - 91%	91%	74% - 94%	95%	87% - 98%
Third	95%	90% - 99%	98%	95% - 99%	98%	92% - 99%

*Includes compounds for which test results were processed by April 10, 1981.

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TABLE 4

NUMBER OF TESTS CONDUCTED AGAINST SELECTED TUMOR SYSTEMS FROM
APRIL 1, 1980, THROUGH MARCH 31, 1981*

Tumor Systems	Second Quarter 1980	Third Quarter 1980	Fourth Quarter 1980	First Quarter 1981	TOTAL
L1210 Lymphoid Leukemia (LL)**	1,936	1,636	1,124	819	5,515
B16 Melanocarcinoma (B1)**	2,754	2,031	1,430	569	6,784
Lewis Lung Carcinoma (LL)**	1,818	1,323	1,013	215	4,369
CDR1 Mammary Tumor (CD)**	1,357	1,855	937	689	4,838
Colon 38 (C38)**	1,632	1,202	761	545	4,140
MSD76 Ovarian Carcinoma (MS)	174	107	41	56	378
CX-1 Colon Xenograft (C2G5)**	756	680	846	836	3,118
CX-1 Colon Xenograft (C2H2)	83	15	47	10	155
LX-1 Lung Xenograft (LKG5)**	663	498	657	573	2,391
LX-1 Lung Xenograft (LKH2)	117	54	30	0	201
MX-1 Breast Xenograft (MRG5)**	572	559	760	799	2,690
MX-1 Breast Xenograft (MRH2)	50	56	27	0	133
TOTAL ALL TUMOR SYSTEMS	11,912	10,016	7,673	5,111	34,712

*Includes tests begun no earlier than April 1, 1980, and reported to the Automated Data Processing (ADP) Contractor by April 10, 1981.

**Panel Tumor Systems

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Table 5

NEW CONFIRMED ACTIVE COMPOUNDS

APRIL 1, 1980 - March 31, 1981

<u>Total Number of Materials</u>	<u>Number of Materials by Tumor Systems</u>
640 <u>in vivo</u>	594 P388 Leukemia 32 L1210 Leukemia 9 B16 Melanoma 2 Lewis lung 1 CD8F1 Mammary tumor 1 Ependymoblastoma 1 Colon 26 carcinoma
9 <u>in vitro</u>	9 9KB5

Table 6

STATUS OF ACTIVE COMPOUNDS

APRIL 1, 1980 - March 31, 1981

New Confirmed Actives:

<u>In Vivo</u>	640
<u>In Vitro</u>	9
Total	649

Compounds Reviewed:

DRS	1,566
DEC	72
Total	1,638

Compounds Passed Decision Network:	23
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TABLE 7

COMPOUNDS SELECTED FOR DEVELOPMENT
 April 1, 1980 - March 31, 1981
 (Decision Network 2A or above)

<u>NSC #</u>	<u>NAME</u>	<u>ASSIGNMENT DATE</u>	<u>ASSIGNMENT BASIS</u>
3051	Formamide, <u>N</u> -methyl-	80-04-30	SRC CX-1; SRC LX-1; SRC MX-1
40774	6-MMPR; 9H-Purine, 6-(methylthio)-9= 8-D-ribofuranosyl-, dihydrate	80-10-29	Brought back for combination clinical studies
65346	Sangivamycin; 7H-Pyrrolo[2,3-d]= pyrimidine-5-carboxamide, 4-amino= 7-8-D-ribofuranosyl-	80-09-10	Returned to DN 2A for development studies; originally selected on L1210
146068	Ed Malonato; Platinum, (1,2-= ethanediamine-N,N')[propanedioato(2-)= -O,O']-, (SP-4-2)-	80-12-10	Recommended by Platinum Analog Committee; B16 melanoma; CD mammary
192965	Spirogermanium hydrochloride; 2-Aza= 8-germaspiro[4.5]decane-2-propanamine, 8,8-diethyl-N,N-dimethyl-, dihydrochloride	80-09-10	Returned to DN 4 level for clinical studies
233853	Adriamycin octanoylhydrazone; Octanoic acid, [1-[4-[(3-amino-2,3,6-trideoxy= α-L-lyxo-hexopyranosyl)oxy]-1,2,3,4,6,11= hexahydro-2,5,12-trihydroxy-7-methoxy-6,= 11-dioxo-2-naphthacenyl]-2-hydroxyethyl= idene] hydrazide, monohydrochloride (2S-cis)-	80-06-12	Recommended by Anthracycline Committee; B16 melanoma; CD mammary

<u>NSC #</u>	<u>NAME</u>	<u>ASSIGNMENT DATE</u>	<u>ASSIGNMENT BASIS</u>
241240	CBDCA; Platinum, diamine[1,1-cyclobut=anedicarboxylato(2-)-O,O']-, (SP-4-2)-	80-12-10	Recommended by Platinum Analog Committee B16 melanoma
253272D	Discreet	81-02-06	Replaces NSC# 262216D as per Operating Committee
256927	Chip; Platinum, dichlorodihydroxybis=(2-propanamine)-, (OC-6-33)-	80-12-10	Recommended by Platinum Analog Committee; B16 melanoma; CD mammary; L1210 leukemia
266210	Bisdaunorubicinhydrazone; Butanedioic acid, bis[[1-[4-[(3-amino-2,3,6=trideoxy- α -L-lyxo-hexopyranosyl)oxy]=1,2,3,4,6,11-hexahydro-2,5,12-trihydroxy=7-methoxy-6,11-dioxo-2-naphthacenyl]=ethylidene]hydrazide], dihydrochloride, stereoisomer	80-06-12	Recommended by Anthracycline Committee; B16 melanoma; CD mammary; Colon 38
267702	Cyclohexyl pyrrolizine derivative; Carbamic acid, cyclohexyl-, [5-(3,4=dichlorophenyl)-2,3-dihydro-1H=pyrrolizine-6,7-diyl]bis(methylene)=ester	80-06-12	B16 melanoma; L1210 leukemia; NCI to supply EORTC with bulk drug
268242	N-Dibenzyl-daunorubicin; 5,12=Naphthacenedione, 8-acetyl-10=[[3-bis-(phenylmethyl)amino]=2,3,6-trideoxy- α -L-lyxo-hexopyranosyl]=oxy]-7,8,9,10-tetrahydro-6,8,11=trihydroxy-1-methoxy-, hydrochloride, (8S-cis)-	80-06-12	Recommended by Anthracycline Committee; B16 melanoma; CD mammary; L1210 leukemia; P388 leukemia

<u>NSC #</u>	<u>NAME</u>	<u>ASSIGNMENT DATE</u>	<u>ASSIGNMENT BASIS</u>
269148	7-Omen; 2,6-Epoxy-2H-naphthaceno[1,2= b]oxocin-9,16-dione, 4-(dimethylamino)-= 3,4,5,6,11,12,13,14-octahydro-3,5,8,10,= 13-pentahydroxy-11-methoxy-6,13-dimethyl-	80-06-12	Recommended by Anthracycline Committee; B16 melanoma; CD mammary; L1210 leukemia
271674	DACH; Platinate(1-), [1,2,4-benzene= tricarboxylato(3-)-O ¹ ,O ²](1,2-cyclo= hexanediamine-N,N')-, hydrogen, (SP-4-3)-	80-12-10	Recommended by Platinum Analog Committee; B16 melanoma; L1210 leukemia
276382D	Discreet	81-03-03	Clinical studies in Japan; B16 melanoma
284356	Gulf Oil Compound; 4,8-Ethenopyrrolo= [3',4':3,4]cyclobut[1,2-f]isoindole= 1,3,5,7(2H,6H)-tetrone, octahydro-	80-09-10	CD mammary; L1210 leukemia
286193D	Discreet	80-10-29	Lewis lung carcinoma
298223	CC 1065; Benzo[1,2-b:4,3-b']dipyrrole= 3(2H)-carboxamide, 7-[[1,6-dihydro-4= hydroxy-5-methoxy-7-[4,5,8,8a-tetrahydro= 7-methyl-4-oxocyclopropa[c]pyrrolo[3,2= e]indol-2(1H)-yl]carbonyl]benzo[1,2-b:= 4,3-b']dipyrrol-3(2H)-yl]carbonyl]-1,6= dihydro-4-hydroxy-5-methoxy-	80-04-30	B16 melanoma
305884D	Discreet	80-12-10	B16 melanoma
325319	Cyclic depsipeptide; Leucine, 1-(2= hydroxy-1-oxopropyl)prolyl-N-methyl= leucylthreonyl-4-amino-3-hydroxy-6= methylheptanoyl-4-hydroxy-2,5-dimethyl= 3-oxohexanoyl-N,O-dimethyltyrosylprol= yl-, ϕ -lactone	80-06-12	B16 melanoma

<u>NSC #</u>	<u>NAME</u>	<u>ASSIGNMENT DATE</u>	<u>ASSIGNMENT BASIS</u>
327471D	Discreet	80-08-19	Replaces NSC# 303565 as per Operating Committee
328564	Quinazoline derivative, Isethionate salt; Ethanesulfonic acid, 2-hydroxy-, compound with 5-methyl-6-[[[3,4,5-trimethoxyphenyl)amino]methyl]-2,4-quinazolinediamine (1:1)	80-11-21	Replaces NSC# 249008 as per Operating Committee
337766D	Discreet	80-12-10	B16 melanoma

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ANNUAL REPORT OF THE PHARMACEUTICAL RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 to September 30, 1981

The Pharmaceutical Resources Branch is structured to provide comprehensive pharmaceutical services to the various programs of the Division of Cancer Treatment. The primary objectives of the Branch are to supply high quality chemical substances and formulated products for investigative program use. These objectives are accomplished essentially through contract support activities. The major contract areas include: chemical preparation and pilot plant production; analytical services; pharmaceutical research and development; and pharmaceutical manufacturing. The synthesis and distribution of radiolabeled chemicals and drugs are also provided through PRB contract sources. Additionally, the Branch is responsible for storage, distribution and computerized inventory maintenance of all drug products used in the Clinical Programs.

TABLE 1

RESOURCE CONTRACTS MONITORED BY THE
PHARMACEUTICAL RESOURCES BRANCH

Chemical Preparation	7
Radiolabel Synthesis	2
Pharmaceutical Production	5
Pharmaceutical Research and Development	2
Analytical Services	2
Drug Distribution	<u>1</u>
Total Resource Contracts	19

The Branch is also responsible for direct purchase of chemicals and drug products utilizing NIH procurement services.

A formulation research laboratory on the NIH campus is operated and staffed by the Pharmaceutical Resources Branch. This laboratory is assigned research projects of high program interest which present difficult drug delivery problems. Most of the chemical agents developed by the Laboratory of Medicinal Chemistry and Biology are assigned to this laboratory. This arrangement facilitates a scientific exchange between the developer and formulator and a team approach to the ultimate product design.

Staff

The Pharmaceutical Resources Branch is presently staffed with six senior professionals, one technical and two secretarial personnel. In addition, one visiting associate and one visiting fellow are assigned to the formulation laboratory. The classification of the senior professionals is as follows: four PHS Commissioned Corps Pharmacists; one Ph.D. analytical chemist; and one Ph.D. medicinal chemist.

The Branch consists of three Sections:

1. Chemical Resources Section - Head, Dr. Robert R. Engle

The primary functions of the Chemical Resources Section are to provide for re-synthesis, large-scale production and procurement services for the acquisition of chemical substances. These services are accomplished by the management and supervision of a contract program for: (a) resynthesis assessment; and (b) pilot plant production of various quantities of bulk substances intended for pharmaceutical manufacture of clinical investigational products. The Section also initiates the acquisition of commercially available chemical substances through NIH procurement contract procedures. This requires the preparation of stringent material specifications to insure that high quality products are obtained.

Another major function of the Section involves the management of a contract program to prepare and distribute radiolabeled materials. These materials are distributed to authorized investigators for clinical pharmacology and other related studies.

This Section supervises seven chemical prep lab contracts and two radiolabel synthesis laboratory contracts.

2. Clinical Products Section - Head, Mr. Larry M. Kleinman

The Clinical Products Section is responsible for production, procurement and distribution of pharmaceutical dosage forms for clinical trial. The activities of the Section involve multi-contract responsibilities and management of several million dollars in drug acquisition purchases.

The major functions of the Section include management of four pharmaceutical production contracts; management of a clinical drug storage, distribution and computerized inventory contract; and the direct purchase of all clinical products used in the various DCT Programs.

Investigational product literature in the form of Investigational Drug - Pharmaceutical Data sheets is prepared by the Section. These information sheets are also supplied in bound book form (NIH Publication No. 81-2141) which is updated periodically. During this reporting period, over 1,000 issues were distributed.

In addition to the management of the pharmaceutical and distribution resource contracts, this Section manages a sizeable intramural budget for the direct purchase of chemicals and formulated products. During this report period, drug purchase expenditures were in excess of 2.5 million dollars. A significant amount of staff time is expended in this area in preparing purchase specifications,

award justifications and periodic budget projections throughout the year. Several types of NIH contract mechanisms are utilized for these procurement actions including bids from suppliers, blanket purchase orders, direct purchase contracts, etc.

3. Analytical and Product Development Section - Head, Mr. James C. Cradock

This Section is responsible for the quality assessment of chemicals and formulated drugs used in the Division of Cancer Treatment's Drug Evaluation Program. Analyses are conducted by contract laboratories independent of chemical or formulated drug suppliers. This Section is also responsible for the development of analytical methodology to determine the purity of chemicals, potency of active ingredients in pharmaceutical formulations, stability of formulated products under accelerated and simulated use conditions, and identification of impurities and/or degradation products. Other evaluations include determination of partition coefficients for use by other DTP components in structure activity studies and application of analytical techniques to estimation of certain compounds in biological fluids. Data are used for preparation of analytical profiles of bulk drugs, designation of reference samples and preparation of purchase specifications of bulk chemicals.

A second major function of this Section involves the development of suitable pharmaceutical dosage forms for clinical trial. Since most preparations are intended for intravenous use, studies are undertaken to assess the solubility and stability in a variety of pharmaceutical vehicles. New approaches to enhance solubility are emphasized since few suitable methods are available to the formulator of parenteral products.

Most of the product development effort is conducted under contract with the Section staff serving as project monitors. In addition, an intramural formulation laboratory supervised and maintained by this Section is investigating methods to solve drug formulation problems.

The Analytical and Product Development Section is responsible for the supervision and management of six contracts: two analytical contracts; two pharmaceutical research and development contracts; one small-scale production contract; and one contract for characterization and evaluation of proteinaceous substances. The protein substances contract is monitored by the Section, but funded by the Biological Response Modifiers Program.

Goals and Accomplishments

The Branch successfully accomplished its objectives during this reporting period by providing efficient chemical and pharmaceutical services to the programs of the Division of Cancer Treatment. For example, the extramural contract program under Branch staff supervision provided over 402 kilograms of synthetic chemical substances for subsequent formulation into investigative clinical products. Among the high priority chemical substances provided include: PALA (NSC-224131), 32 Kg; D-amygdalin (NSC-15780), 131 Kg; D,L-amygdalin (NSC-251222), 59 Kg; Methyl GAG (NSC-32946), 17 Kg; and Desmethylnisonidazole (NSC-261036); Dihydro-5-azacytidine (NSC-264880); ICN nucleoside (NSC-286193); and others.

In addition to the delivery of large batch preparations, the Chemical Resources Section assigned 128 panel compounds for resynthesis. The delivery and inventory of panel compounds was coordinated with the Drug Synthesis and Chemistry Branch and an effective monitoring system for these substances now exists. During this reporting period, the Chemical Resources Section also monitored two radiosynthesis contracts with procurement of 24 radiolabeled substances.

The Analytical and Product Development Section continued to provide superior analytical contract services to the program. In addition to the evaluation of chemical and formulated products, the analytical contractors assisted the Natural Products Branch with analytical characterization of substances they received. The Drug Synthesis and Chemistry Branch has also been assisted with similar problems of analytical characterization and evaluation.

The Analytical and Product Development Section developed improved analytical methods for Amygdalin and Tetrahydrocannabinol. Work is in progress on the improvement and development of optimal human delivery systems for the cannabinoids which are of high program interest as antiemetics for chemotherapy induced nausea and vomiting. The completion of an experimental formulation of Spirohydantoin Mustard (NSC-172112) was also accomplished utilizing a complex vehicle system. Eight publications resulted from the laboratory efforts of this Section.

The pharmaceutical production activity has successfully maintained a clinical inventory of high quality investigational products for the Division of Cancer Treatment Program. The contractors under the supervision of the Clinical Products Section produced over 600,000 injectable units and over three million oral units for clinical trial. Several products of continued high interest include: AMSA, AZQ, Azacitidine, Methotrexate and Hexamethylmelamine.

The Quality Control Committee for Bulk Chemicals and Drugs, which is comprised of senior staff members in the Branch, played a key role in reviewing analytical and manufacturing reports on bulk items and formulated items. This Committee has invested considerable effort in developing product specifications for new investigational materials.

The Branch continues to meet all new challenges with interest and enthusiasm. Our objective is to be prepared to meet all challenges and to maintain a contract capacity to provide efficient and productive pharmaceutical support to the Division of Cancer Treatment Program. Two major clinical programs that will involve significant Branch coordinated contract efforts include the national Tetrahydrocannabinol (THC) antiemetic program and the clinical trials program with Amygdalin. Both programs will involve large-scale projects for the chemical preparation and pharmaceutical production contracts. A product development effort for improving bioavailability of THC dosage forms will be continued in the formulation laboratory. The clinical distribution of THC as a controlled substance presents significant logistical problems which are currently being assessed with the Cancer Therapy Evaluation Program.

A problem of continued concern is the increasing number of poorly soluble compounds, particularly natural products, that when solubilized show a loss of tumor activity. Several such compounds showing good antitumor activity when administered in suspension (i.p./i.p.) exhibit greater toxicity and diminished activity when solubilized and administered intravenously. This problem will be given

considerable attention during the next year with the hope of increasing the number of new agents for clinical trial.

In order to comply with all Food and Drug Administration regulations for investigational drugs, a new contract project is being sought for the shelf life surveillance of all clinical products. This contract will be monitored and supervised by the Analytical and Product Development Section and the reports from the contractor will be filed with the FDA as required.

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Beltagy, Y.A., Waugh, W., and Repta, A.J.: Formulation and stability of the antineoplastic agent n,n'-di-9-acridinyl-1,6-hexanediamine (NSC-219733). Drug Develop. Ind. Pharm. 6: 411-421, 1980.

CHEMICAL RESOURCES SECTION
PHARMACEUTICAL RESOURCES BRANCH

Scope

The function of the Chemical Resources Section is to procure bulk chemicals and drugs for confirmational and other testing, toxicological and pharmacological evaluation, and clinical trials in humans; and to procure radiolabeled materials for pharmacology, clinical pharmacology and related studies. The function of the Section is achieved through the management and supervision of a contract program for the preparation of various quantities of bulk chemicals and drugs and radiolabeled materials, and the acquisition of commercially available chemical substances.

Staff

The staff of the Chemical Resources Section consists of one professional person and a secretary. The professional staff member serves as the project officer on the various contract activities of the Section.

Preparative Activities

The resynthesis or preparation laboratories are, in the strictest sense, service laboratories and are designed and selected to prepare known chemicals and bulk drugs which are needed by the Program. The compounds selected for preparation are not readily available in the quality or quantities needed from the original supplier or on the open market.

These laboratories are also used to obtain data for the preparation of the necessary quantities of clinically important chemicals and to develop the most economical means for their preparation. It should be pointed out that many methods of synthesis which are practical for small quantity are not technically feasible or economically practical when used for a large-scale synthetic operation or for radiolabel synthesis. The conversion of small-scale to large-scale production often requires developmental studies which are conveniently carried out by the preparation laboratories. Solubility and stability studies and cost data are also provided by these contractors.

The increased emphasis in clinical investigations has caused a change in the preparation activities. The increased effort being devoted to toxicological and clinical studies has resulted in the need for larger quantities of the compounds prepared by this program. Also, the decision to evaluate a larger number of compounds demonstrating confirmed activity in the initial screening in the tumor panel has also resulted in the need for larger quantities of the compounds being synthesized for preclinical evaluation. This change in emphasis has been handled by the preparation laboratories without difficulty.

The preparation laboratories, taken collectively, provide the means of obtaining nearly any type of chemical compound, regardless of structure, and the ability of providing large quantities of very high purity drugs.

1. Bulk Chemicals and Drugs

The compounds chosen for resynthesis at the preparation laboratories are assigned for five major reasons: (1) clinical investigations; (2) preclinical toxicological and pharmacological evaluation; (3) maintaining a stock of chemicals of clinical and preclinical interest; (4) completion of screening or confirmational testing; and (5) use as intermediates in further synthesis. The compounds so chosen are placed on priority lists based upon their relative importance, with clinical compounds given top rating. The quantity of a given material to be resynthesized may vary from one gram to multikilograms. Factors governing the amounts depend upon: use, ease of preparation, stability, and cost. Materials of high quality are prepared in the preparation laboratories. Every effort is made to safeguard the patient in the quality of the drugs chosen.

The Chemical Resources Section, through the preparative laboratories, directed the resynthesis of 257 compounds totaling 402 kilograms during this report period. Included in this group were 23 compounds scheduled for or undergoing clinical evaluation totaling 344 kilograms and 128 panel compounds. In addition to the compounds resynthesized, 52 new compounds were also prepared by the preparative laboratories. The Section also purchased nine compounds scheduled for or undergoing clinical evaluation totaling more than 237 kilograms. Quantities of three key intermediates for clinical compounds were purchased during this report period totaling 133.5 kilograms.

During this report period, large quantities of Methyl CCNU (NSC-95441) (22 Kg); N-methylformamide (NSC-3051) (10 Kg); Methyl G (NSC-32946) (17 Kg); Cycloleucine (NSC-1026) (43 Kg); PALA (NSC-224131) (32 Kg); D-amygdalin (NSC-15780) (131 Kg); and D,L-amygdalin (NSC-251222) (59 Kg), were prepared by the preparation laboratories. The D-amygdalin was obtained from the extraction of ten tons of apricot kernels. In addition, the preparation laboratories responded rapidly to the preparation of large amounts of several other high priority materials needed by the Program such as NSC-261036 (Desmethylnisonidazole); NSC-301467 (the SRI radiosensitizer, SR-2508); NSC-264880 (Dihydro-5-azacytidine); NSC-139490 (5-Methyltetrahydrohomofolic Acid); and NSC-286193 (the ICN nucleoside).

One of the preparation laboratories, Aerojet Strategic Propulsion Company, has worked on the development of a feasible and practical procedure for the preparation of trans-Delta-9-tetrahydrocannabinol (NSC-134454) (THC) on large (kilogram) scale. The approach investigated is a modification of the Razdan one-step procedure with purification via industrial scale gas chromatography. The feasibility of this approach has been demonstrated and the production of this compound via this approach is in progress.

The Section directed seven contract laboratories for the preparation of materials needed by the Program (Table 2).

TABLE 2

CONTRACT LABORATORIES FOR PREPARATION OF CLINICAL AND EXPERIMENTAL COMPOUNDS

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Aerojet Strategic Propulsion Company	Olsen	N01-CM-17490

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Aldrich Chemical Company	Szabo	N01-CM-17492
Ash Stevens, Inc.	Markovac	N01-CM-17488
Monsanto Research Corporation	Ellard	N01-CM-97255
Pharm-Eco Laboratories, Inc.	Draper	N01-CM-17487
Starks Associates, Inc.	Parsons	N01-CM-17374
Warner-Lambert Company	Cook	N01-CM-17491

2. Radiolabeled Materials

The Section coordinates the procurement and distribution of radiolabeled chemicals and drugs for pharmacological and drug distribution studies. Materials not available from commercial sources are prepared by contract preparative laboratories. These contracts also handle the storage and distribution and perform the necessary analytical work for all labeled materials, whether prepared under the contracts or acquired from other sources. All requests for radioactive materials are reviewed by a committee in relation to proposed use prior to assignment to the contracts for procurement and/or shipment. All materials are checked for purity prior to shipment and repurified, if necessary, before shipping. A total of 24 radiolabeled materials have been procured from all sources. Of these, 19 were ¹⁴C labeled compounds prepared by the two radiosynthesis contractor laboratories including DON (NSC-7365); DHAQ (NSC-279836); AZQ (NSC-182986); AMSA (NSC-249992); Adriamycin (NSC-123127); WR-2721 (NSC-296961); the Isopropyl Pyrrolizine Derivative (NSC-278214); Hexamethylmelamine (NSC-13875); Methyl G (NSC-32946); and Aphidicolin (NSC-234714). One hundred sixty-six shipments of radioactive substances were made during this report period.

The Section directed two contracts devoted to the procurement of radiolabeled materials (Table 3).

TABLE 3

CONTRACT LABORATORIES FOR PREPARATION OF RADIOLABELED MATERIALS

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Research Triangle Institute	Kepler	N01-CM-97313
SRI International	Leaffer	N01-CM-97256

CLINICAL PRODUCTS SECTION
PHARMACEUTICAL RESOURCES BRANCH

Scope

The responsibilities of the Clinical Products Section include the following:

1. Production, packaging and labeling of investigational dosage forms;
2. Procurement by direct purchase of formulations for clinical use;
3. Shelf life surveillance of all clinical dosage forms;
4. Preparation of FDA-IND attachments 1, 2, 3, 4, 5 and 7 for subsequent submission to the Food and Drug Administration;
5. Preparation of Investigational Drug - Pharmaceutical Data sheets for Clinical Brochures, IND's, etc.;
6. Management of a computerized clinical drug inventory system; and,
7. Storage and distribution of clinical dosage forms.

Staff

The staff of the Clinical Products Section consists of two senior professionals and one purchasing agent. The professional staff members are PHS Commissioned Corps Pharmacists and serve as project officers on the various contract activities of the Section.

Production and Packaging of Investigational Drugs

The Section has the responsibility to provide adequate quantities of formulated drugs to the Clinical Programs. The production of investigational dosage forms is mainly performed by contract agreements with qualified pharmaceutical firms. The production output of investigational dosage forms for the period covered by this report remained at a high level of effort. The formulation contractors produced a combined total of 591,525 parenteral doses and 74,271 bottles of tablets and capsules (3,153,150 oral doses). Production output which is dictated by clinical needs remained constant during this report period. The production contractors have been able to meet the Program's needs for a variety of formulated products. Five contracts were monitored by the Clinical Products Section.

TABLE 4

CONTRACTS MONITORED BY THE CLINICAL PRODUCTS SECTION

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Ben Venue Laboratories, Inc.	Wickes	NO1-CM-97298

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Elkins-Sinn, Inc.	Reese	N01-CM-67103
Flow Laboratories, Inc. ¹	Miller	N01-CM-67088
Philips Roxane Laboratories, Inc.	Marine	N01-CM-67053
Yamanouchi Pharmaceutical Co., Ltd.	Kawata	N01-CM-97307

¹Responsible for clinical drug storage and distribution and operation of a computerized clinical drug inventory system.

Several investigational products are provided by pharmaceutical companies on a cost-sharing basis. These products are usually provided in return for access to clinical data generated by the studies. The dosage forms are prepared from specifications which are established by mutual agreement between the Branch and the manufacturers. During the period of this report, the following companies supplied a total of 38,827 parenteral units as well as 143,100 tablets and capsules as part of this joint effort:

Adria Laboratories (USA)	NSC-246131	AD-32
Dome Laboratories (USA)	NSC-178248	Chlorozotocin
Hoffmann-La Roche (USA)	NSC-261037	Misonidazole
Janssen Pharmaceuticals (USA)	NSC-177023	Levamisole
Lederle Laboratories (USA)	NSC-301739	Dihydroxyanthracenedione
Pfizer Inc. (USA)	NSC-331615	Levonantradol
Rhone-Poulenc Inc. (France)	NSC-164011	Rubidazone
Wellcome Foundation (England)	NSC-220537	Corynebacterium Parvum

Purchase of Dosage Forms

Another function of the Section involves the direct purchase of formulated products. These items fall into two categories: (1) investigational products; and (2) commercial products. Investigational products are usually manufactured according to NCI specifications and sold to NCI on a cost-reimbursement basis. Commercial products are items available for open purchase. Procurement of all direct purchased dosage forms is handled through the NIH Procurement Branch. Several procurement mechanisms are used depending on such factors as the type of product, the quantity required and the priority for clinical need.

A total of 232,215 injectable units and 730 bottles of tablets and/or capsules (197,340 oral doses) of direct purchased items, at a cost of \$2,121,258, were obtained during this report period. An additional sum of \$338,169.10 was used for the acquisition of bulk chemicals and natural products. All suppliers are

required to provide a certificate of analysis on each lot of drug purchased. Bulk chemicals and natural products are assayed by private contractors to ascertain whether or not requested specifications are met. The data are reviewed prior to release for clinical use or formulation, respectively.

The table below summarizes the procurement activities of the Clinical Products Section.

TABLE 5
DRUG ITEMS PROCURED

	<u>Capsules/Tablets</u>	<u>Injectables</u>
Produced by Contractors	3,153,150	591,525
Contributed or Cost-Sharing	143,100	38,807
Purchased Investigational Units	0	142,200
Purchased Commercial Units	<u>52,250</u>	<u>232,215</u>
	3,348,500	1,004,747

The expenditures for commercially available drugs were lower in FY-1981 because of supplemental funding provided in the third quarter of FY-1980. The funds were used to obtain additional supplies of commercial drugs and were of sufficient magnitude to permit a smaller appropriation in FY-1981. The supplement provided for 462,813 injectable commercial units and 36,632 bottles of tablets and/or capsules totaling 3,427,840 solid oral dosage forms.

The Clinical Products Section is responsible for the disbursement of funds from Common Account Number (CAN) 1-8322759. This account is used for the acquisition of products intended for clinical trial which include natural products, bulk chemicals and clinical products. Careful monitoring of procurement is essential since the operational budget of \$2,050,000 (FY-1981) is below the projected requirement.

Quality Assessment of Clinical Dosage Forms

Each lot of formulated drug must pass quality assessment criteria prior to release for clinical trials. The Clinical Products Section, in collaboration with the other two Sections in the Branch, maintains a strict assessment program designed to insure that each batch of formulated material meets the criteria for strength, quality and purity. Parenteral products are tested for potency, sterility, safety, pyrogenicity, etc., while oral formulations are tested for potency, content uniformity, weight variation, hardness, disintegration, dissolution, etc. All products are tested for potency by the formulator with random testing being conducted by an analytical contractor. All new dosage forms and all first production batches of a new formulator are routinely submitted to an analytical contractor to verify the accuracy of the formulator.

The pharmaceutical contractors are requested to perform compatibility and stability studies on reconstituted and diluted solutions of parenteral investigational dosage forms. These studies are designed to simulate the conditions found in the clinical setting. The data generated is assembled and is summarized in the format of an Investigational Drug - Pharmaceutical Data sheet which is incorporated into the Clinical Brochure.

The Clinical Products Section maintains a shelf life surveillance program on all lots of contractor produced investigational dosage forms. Random samples of each lot are stored under specified controlled conditions and are assayed for chemical and/or physical change. The stability schedule has been modified to conform to guidelines proposed by the Food and Drug Administration to establish an expiration date for a product. Three lots of a product will be followed under accelerated conditions (Schedule A). These lots will be followed for a period of four years. In addition, a sampling of each lot of drug produced by a contractor will be retained for two years beyond its expiration data. This will insure that an adequate number of samples are available to repeat any testing required.

Storage and Distribution of Clinical Dosage Forms

The Clinical Products Section administers a contract for the storage and distribution of clinical drugs. During 1980, the contractor prepared almost 13,000 drug shipments--an average of 51 shipments each day. This represents approximately 49,000 bottles of tablets and capsules and almost one million vials and ampules for the year. As the implementation of the THC Program has progressed over the last several months, the volume of drugs distributed has been impacted. The average number of orders has increased 20% to approximately 60 each day and is still climbing.

To provide efficient processing of orders and also to provide the necessary inventory control, the computerized Drug Distribution Inventory System has processed each of the 13,000 orders, imputing the required information into the data bases. A total of 485 reports (mostly the routine daily drug inventories) were generated during 1980. Of these, 30 were special one-time queries for such items as recalls due to IND closings, cost determinations for specific drugs or specific investigators, and during the past year, to answer numerous questions from the General Accounting Office (GAO). Additionally, 16 separate improvements to the computer program were implemented.

Preparation of Investigational Drug - Pharmaceutical Data Sheets

The Clinical Products Section is responsible for the preparation of pharmaceutical data sheets for all investigational dosage forms used in the Program. The data sheets are used in the preparation of Clinical Brochures and are compiled and distributed to health-care practitioners in a book entitled "NCI Investigational Drugs - Pharmaceutical Data". These data sheets provide product descriptions, reconstitution and stability information, and precautions in handling the products. These data sheets are updated as new stability data become available.

FDA-IND Submissions

The Clinical Products Section also prepares Investigational New Drug (IND) Attachments 1, 2, 3, 4, 5 and 7 (labels) for submission to the Investigational

Drug Branch, CTEP, DCT, NCI, for FDA-IND filing. These attachments contain manufacturing, control, packaging and labeling data required for the investigational dosage forms. During this report period, the Section prepared attachments for several original IND's and numerous amended IND's.

Significant Accomplishments

The pharmaceutical production contractors of the Section were able to meet an increased clinical demand for several high interest drugs.

The production contractors produced over 84,883 vials of Cisplatin (10 mg and 25 mg); over 56,926 vials of Methotrexate (1.0 Gm and 50 mg); 38,805 vials of Daunorubicin; 36,368 vials of Calcium Leucovorin; and 44,680 vials of Azacitidine. In addition, there were several drugs that, because of their poor water solubility or stability, required the preparation of one or two special diluents. The contractors produced 42,456 vials of AZQ and diluents; 38,820 vials of PCNU and diluents; and 57,346 vials of Amsacrine and diluent. These multicomponent products required special packaging and extensive label design.

The Clinical Products Section has continued to develop a dialog with pharmacists, physicians and other health-care practitioners participating in clinical oncology programs at the various cancer centers. This dialog has resulted in a timely exchange of information beneficial to both parties. This information is especially important with drugs undergoing Phase I and Phase II clinical trials.

This activity involved the distribution of over 1,000 NCI Investigational Drug - Pharmaceutical Data books and the handling of 20-25 inquiries per week. During FY-1981, the Section had the book revised. The response from the field has been very enthusiastic.

During FY-1981, the Division of Cancer Treatment placed Delta-9-tetrahydrocannabinol (THC) into guideline distribution to control the nausea and vomiting resulting from chemotherapy. This program directly impacted on the Section in several ways. The storage contractor was required to construct a vault suitable for the storage of Schedule I controlled substances. This was accomplished in less than 60 days. The Section was responsible for the transportation of the initial inventories of THC from NIDA's contractor to the Section's storage and distribution contractor. The Section also directed the manufacture of four large batches of various strengths of THC capsules. The Section devised new label formats utilizing NCI's standard design. Since all the NIDA products of THC were identical in color and shape, the Section initiated a change in the color of the 5 mg capsule strength to reduce the possibility of medication error. The Section also initiated its own contract for the manufacture of soft gelatin capsules.

The Section had to devise several difficult labels for Interferon and the double-blind study of Laetrile. This was accomplished in an expedient manner.

ANALYTICAL AND PRODUCT DEVELOPMENT SECTION

PHARMACEUTICAL RESOURCES BRANCH

Scope

The analytical chemistry and dosage form development activities are merged into one Section within the Pharmaceutical Resources Branch. Major responsibilities of the Section fall into two distinct categories:

1. Analytical Chemistry

- a. Develop suitable methodology for the analysis and characterization of bulk chemicals and pharmaceutical dosage forms.
- b. Assess the quality of bulk chemicals and pharmaceutical dosage forms used in the Developmental Therapeutics Program.
- c. Develop specifications for use in procurement of bulk chemical agents.
- d. Serve as a repository for all methods of preparation and analytical characterization of bulk chemicals. The data are summarized in a Chemical Information Sheet and made available to investigators using these materials.

2. Dosage Form Development

- a. Characterize the pharmaceutical properties of new chemical agents, develop dosage forms and evaluate any chemical and/or physical changes under simulated use conditions.
- b. Develop new approaches for enhancing solubility and stability of selected antitumor agents.

Staff

This Section is staffed by one pharmacist, one analytical chemist, a visiting associate and a visiting fellow.

Analytical Chemistry

The development and preparation of drug products for clinical investigation requires the support of extensive analytical testing. Rigorous assessment of identity and purity beginning with the bulk chemical and continuing through to the finished dosage form is essential to insure that these materials are of high quality. When a bulk chemical is received for development, analytical methods are devised by the contract laboratories to confirm the identity as well as to evaluate the purity of the material. Solubility and stability studies are performed to provide information to aid in dosage form development. After analytical data have been compiled on several lots, bulk chemical specifications are established to aid in the quality control of these materials. These specifications

are also used as minimum acceptance criteria for bulk chemicals purchased in the open market on a bid basis.

The pharmaceutical dosage form is similarly examined to establish identity and purity. In addition, content uniformity and weight variation tests may be performed as described in the United States Pharmacopeia XX.

Analytical data compiled on the bulk drug substance and the pharmaceutical dosage forms are submitted to the Food and Drug Administration as a part of IND applications filed by the National Cancer Institute.

In addition, information acquired from these analytical laboratories is condensed into a "Chemical Information Sheet". This compilation is a part of the Clinical Brochure which is distributed to the various clinical investigators. The data provide information on the characterization of the drug substance, serve as a guide for determining storage and shipping procedures and supply preliminary chemical and solubility information for use by investigators involved in other aspects of drug development.

The determination of the purity of a new chemical entity is often a complex problem. Consideration must be given to drug stability and the methodology developed must be capable of determining the drug in the presence of decomposition products or impurities. Methods commonly used include: spectroscopy (ultraviolet, visible infrared and nuclear magnetic resonance); chromatography (paper, thin layer, high pressure liquid and gas liquid); elemental analysis; and, when appropriate, optical rotation, refractive index, Karl Fischer water analysis, etc.

During this year, 175 lots of bulk chemicals and clinical formulations were evaluated by the contract laboratories for identity and purity. Also, numerous stability and solubility studies were completed on bulk drugs. In addition to the more routine analytical assignments, additional specific projects are occasionally necessary. One contractor, SRI International, performed additional studies in response to FDA inquiries regarding analytical data included in IND filings (most notably Amygdalin). In the case of Amygdalin, a prompt and effective response avoided an interruption of the ongoing clinical trials. The other analytical contractor, Midwest Research Institute, devoted considerable effort to the analysis of bulk and formulated Delta-9-tetrahydrocannabinol presented unique problems and have required the development of suitable analytical methodology.

Analytical services are provided by the two analytical-quality control contract laboratories listed in Table 6. Also, elemental analyses are obtained under a purchase agreement from Micro-Analysis, Inc. of Wilmington, Delaware. These services are essential to insure that high quality pharmaceuticals are provided for NCI sponsored clinical trials and to meet the increasingly detailed and complex Federal regulations applicable to all sponsors of clinical investigations of pharmaceuticals. Also, a small contract has been established with South Mountain Laboratories, Inc. to provide additional sterility, pyrogen and safety test capabilities to the Pharmaceutical Resources Branch. This contract enables PRB to independently conduct sterility and pyrogen tests on contractor produced and purchased materials in the same manner used for chemical assessment of bulk chemicals and pharmaceutical dosage forms.

TABLE 6

CONTRACT LABORATORIES FOR ANALYTICAL CHEMISTRY

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Midwest Research Institute	Helton	N01-CM-87234
SRI International	Lim	N01-CM-87183

Dosage Form Development

Dosage form development studies are usually limited to potential antitumor agents that meet Decision Network 2A criteria. However, product development studies have been carried out during the past year in two cancer related areas: (1) development of bioavailable dosage forms of the antiemetic Delta-9-tetrahydrocannabinol and the formulation of the Interferon inducer Poly ICLC.

Since biologically suitable solubilization techniques are limited, new approaches are investigated under contract at the University of Kansas and at the University of Kentucky as well as in the Pharmaceutical Resources Branch's formulation laboratory (see Project Z01-CM-03584-09 PRB). During the past contract year, the contract resources in the formulation research area were doubled with the addition of the two man year effort at the University of Kentucky. This change was made in response to the increase in the number of DN2A candidate compounds that exhibited inadequate solubility and/or stability.

The first approach to development involves an evaluation of existing physical and chemical data as well as in vivo route and regimen studies in rodents. These results serve to define the scope of the formulation problem. Relatively straightforward development projects are assigned directly to the contract laboratory equipped with production capabilities. The contractor is provided with such information as chemical data, estimated dose and intended route of administration. Consultation with the project officer is carried out regarding formulation approaches. The chemical is subjected to solubility and stability studies in a selected group of physiologically acceptable vehicles. An analytical method (usually HPLC) is devised for detection of the parent compound in the presence of excipients and/or degradation products. The influence of pH, temperature, light, oxygen, packaging components and pharmaceutical additives is assessed on the stability of drug solutions. Pilot batches are then prepared under conditions simulating production scale. Physical and chemical stability of the dosage form is assessed under accelerated and simulated use conditions.

Problem areas in parenteral dosage form development directed to intravenous administration are generally due to inadequate water solubility or instability of the agent of interest.

The University of Kansas studied four compounds during the past year and successfully developed soluble forms for JB-11 (NSC-249008); the isopropyl Anderson Compound (NSC-278214); and Valinomycin (NSC-122023). An emulsion was used in the latter two cases to achieve adequate solubility. NSC-278214 is exceedingly unstable in aqueous solutions ($T_{1/2} \leq 15$ minutes). Incorporation in the emulsion increased the useful stability period (T_{90}) to at least two hours.

In last year's Annual Report, we discussed at some length a problem that infrequently occurred when conducting a bulk versus formulated antitumor comparison on drugs that are inherently poorly water soluble. In a number of instances, activity was either lost or severely diminished when administered I.P. versus I.P. transplanted tumor models. This problem still exists, but did not occur with the previously observed frequency. However, the experience with Valinomycin should be described. When administered in the emulsion system, the toxic dose was reduced 20-40 fold and the activity reduced in half. The onset of toxicity was within five to 60 minutes in most cases and was presumably due to release of potassium ion into the systemic circulation. These data demonstrate a significant bioavailability problem with I.P. Valinomycin suspensions, absence of substantial antitumor activity particularly by the intravenous route, and the suggestion of a significant and poorly manageable toxicity that renders doubtful further development of this compound.

The University of Kentucky has also worked on four drugs and developed useful formulations of NSC-241240, a Platinum analogue; and NSC-212509, B-Hydroxywithanolide E. Some interesting complexation work was completed using the vitamin nicotinamide that may be useful for other compounds.

The University of Iowa contract provides both sophisticated product development capability and production capacity for sterile freeze dried dosage forms, small- and large-scale sterile solutions plus tablets and capsules for oral use. The production capacity is usually adequate to meet requirements for large animal toxicity testing or Phase I clinical trial. During the past year, they have developed formulations for NSC-280594, Tricyclic nucleoside 5'-phosphate; NSC-262666, Pettit's mustard; NSC-40774, 6MMPR; and NSC-301467, a radiosensitizer. Initial production batches were prepared for NSC-280594, Ellipticine tablets, 6MMPR and the Henkel Compound. Dosage form development and manufacturing data are used in support of IND Applications. These projects reflect the versatility of this contractor in dosage form development, scale up and production. During the past year, five development and 15 production projects were completed.

TABLE 7

CONTRACTS FOR DOSAGE FORM DEVELOPMENT

<u>Contractor</u>	<u>Investigator</u>	<u>Contract No.</u>
Iowa, University of	Wurster	N01-CM-07303
Kansas, University of	Repta	N01-CM-07304
Kentucky, University of	Hussain	N01-CM-07381

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03584-09 PRB																														
PERIOD COVERED October 1, 1980 to September 30, 1981																																
TITLE OF PROJECT (80 characters or less) Research in the Development of New Antitumor Drugs																																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">James C. Cradock</td> <td style="width: 20%;">Head</td> <td style="width: 10%;">A&PDS</td> <td style="width: 10%;">PRB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>J. Paul Davignon</td> <td>Chief</td> <td></td> <td>PRB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Karl P. Flora</td> <td>Chemist</td> <td>A&PDS</td> <td>PRB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Babu R. Vishnuvajjala</td> <td>Visiting Associate</td> <td>A&PDS</td> <td>PRB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Gururaj Bykadi</td> <td>Visiting Fellow</td> <td>A&PDS</td> <td>PRB</td> <td>NCI</td> </tr> </table>			PI:	James C. Cradock	Head	A&PDS	PRB	NCI	Other:	J. Paul Davignon	Chief		PRB	NCI		Karl P. Flora	Chemist	A&PDS	PRB	NCI		Babu R. Vishnuvajjala	Visiting Associate	A&PDS	PRB	NCI		Gururaj Bykadi	Visiting Fellow	A&PDS	PRB	NCI
PI:	James C. Cradock	Head	A&PDS	PRB	NCI																											
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	Gururaj Bykadi	Visiting Fellow	A&PDS	PRB	NCI																											
COOPERATING UNITS (if any) Dr. C. Litterst, Laboratory of Medicinal Chemistry and Biology, NCI Dr. E. Perlin, Dept. of Clinical Pharmacology, USUHS Dr. L. McCarthy, Dept. of Pharmacology, Dartmouth Medical College																																
LAB/BRANCH Pharmaceutical Resources Branch																																
SECTION Analytical and Product Development Section																																
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																																
SUMMARY OF WORK (200 words or less - underline keywords) This project describes the <u>formulation</u> activities in the intramural laboratory with drugs of interest to the <u>Division of Cancer Treatment</u> , NCI. Primarily, these studies are directed toward evaluation of the stability of certain new antitumor agents and resolution of solubility problems presented by these substances. <u>NSC-D286193</u> ; <u>NSC-284356</u> , 4,8-Ethenopyrrolo(3',4':3,4)cyclobut(1,2,-F)-isoindole-1,3,5,7(2H-6H)-tetrone, octahydro-; and <u>NSC-271674</u> , 4-Carboxy-phthalato-(1,2-diaminocyclohexane) platinum are representative examples. Formulation studies were not restricted to antineoplastic agents and included formulation studies on the antiemetic <u>Delta-9-tetrahydrocannabinol</u> designed to improve bioavailability of the oral dosage form and provide alternative formulation approaches for administration by intravenous, intramuscular and rectal routes.																																

1. Delta-9-tetrahydrocannabinol, NSC-134454 (Flora, Cradock, Vishnuvajjala and Litterst, LMCB)

Nausea and vomiting have long been associated with aggressive cancer chemotherapy. Delta-9-tetrahydrocannabinol (Delta-9-THC) has been shown to control the nausea and vomiting in many patients including some whose symptoms were refractory to standard antiemetic therapy. Absorption of Delta-9-THC from the dosage forms currently available from the National Institute on Drug Abuse appears to be erratic and unpredictable. Therefore, studies were undertaken to provide a variety of bioavailable dosage forms of THC.

Current investigations have concentrated on several approaches including: development and bioavailability testing of parenteral dosage forms; development of bioavailable oral formulations; and development of dosage forms for rectal administration.

Solutions containing at least 10 mg/ml Delta-9-THC have been prepared for parenteral use in each of two vehicles: 5% Cremophor EL-5% ethanol-90% Sodium Chloride Injection or 5% Emulphor EL620-5% ethanol-90% Sodium Chloride Injection. Bioavailability of radiolabeled Delta-9-THC in the Emulphor EL620 vehicle administered intramuscularly has been studied in rabbits (Dr. Litterst, LMCB). After intravenous administration, the plasma decay curves (total radioactivity) were characterized by three distinct phases with half-lives of 36, 538 and 1287 minutes. Intramuscular administration of THC in the emulphor vehicle produced prolonged peak levels of radioactivity (15-30 hours). I.M. administration produced an Area Under the Curve about one-third of the I.V. value.

Collaborative studies of the emulphor formulation in the feline emesis model of Borison and McCarthy have been conducted (McCarthy, Dartmouth Medical School). Cisplatin is used to induce emesis in this procedure. A radioimmunoassay kit available from the National Institute on Drug Abuse for the determination of Delta-9-THC in human plasma was adapted in our laboratory for application to cat plasma and used to monitor plasma THC. Numerous courses and doses of Delta-9-THC in the emulphor formulation have been administered intramuscularly or intravenously to cats.

Peak plasma levels were achieved two to six hours after intramuscular administration of THC. As with the rabbits, the decay of plasma THC was prolonged after I.M. administration. An intramuscular dose of 2.0 mg/kg gave substantial protection against Cisplatin induced emesis. Future studies will use higher I.M. doses and a variety of new oral formulations. Several oral formulations for possible administration in soft gelatin capsules have been prepared. In vitro release characteristics have been studied using the USP XX (paddle) dissolution method. Dissolution samples were assayed by gas-liquid chromatography. Vehicles evaluated include: 10 or 20% Emulphor EL620 in sesame oil; 10 or 20% Emulphor EL620 in PEG 400; 10 or 20% Cremophor EL in PEG 400; 10 or 20% Emulphor EL620 in oleic acid; and 10 or 20% Cremophor EL in oleic acid. In vitro release of Delta-9-THC from these vehicles varied from moderate to good; but, in all cases, the release was better than from sesame oil alone, the vehicle for the formulation currently in clinical use.

Other collaborative investigations have been conducted with Drs. Perlin and Smith of the Uniformed Services University of the Health Sciences in the monkey. The

results indicate that I.M. THC in the Emulphor formulation is also well absorbed in this species. We have prepared and evaluated several Delta-9-THC containing suppositories. Typical suppository compositions were: 10 or 20 mg of delta-9-THC in the following bases: cocoa butter, 15% Cremophor EL in cocoa butter, 20% cetomacrygol in cocoa butter, 5% cetomacrygol-15% Cremophor EL in cocoa butter, 15% Cremophor EL in cocoa butter, 15% Cremophor EL-21% PEG 4000 in PEG 1500 and others. In vitro release of Delta-9-THC was measured using the USP XX paddle method. The base containing the PEG's demonstrated the best release characteristics for THC. However, all bases containing surfactants demonstrated better in vitro release than did the cocoa butter base alone. Unfortunately, administration of either the cocoa butter or Cremophor-PEG based suppositories containing THC to monkeys produced little or no plasma THC levels.

2. Taxol, NSC-125973 (Bykadi, Flora and Cradock)

During the past few years, difficulty was occasionally encountered in confirming the activity of soluble dosage forms relative to the same drug in suspension in tumor models using i.p. treatment of an i.p. tumor transplant. One such example is the plant product Taxol that exhibits good activity (% ILS > 100%) versus i.p. B₁₆ melanoma when administered i.p. as a suspension. Taxol is a poorly water soluble compound (about 10 µg/ml). Solubility adequate for antitumor evaluation as a solution could be achieved in 75% polyethylene glycol or in a mixture of Cremophor-ethanol-saline, 5:5:90. However, confirmatory antitumor testing repeatedly demonstrated diminished antitumor responses when compared to a suspension of Taxol in Klucel. These data suggest that the diminished antitumor response observed with soluble forms may be due to more rapid absorption from the i.p. cavity into the systemic circulation resulting in diminished drug concentration i.p. Also of concern was the potential for inactivation in plasma due to hydrolysis by plasma esterases. These hypotheses assume that a substantial portion of the i.p. antitumor response versus i.p. B₁₆ is due to a topical effect resulting from high i.p. drug concentrations.

This aspect was evaluated indirectly by monitoring plasma levels following i.p. administration of Taxol in suspension, 75% PEG solution and in Cremophor-ethanol-saline solution. Suitable extraction and analytical methods were developed using ethyl acetate to extract Taxol from plasma with 90-95% efficiency. The extract was subsequently dried, then dissolved in mobile phase and analyzed by HPLC using U.V. detection. Preliminary results from nontumor bearing animals demonstrate an inverse relationship between peak plasma levels and the antitumor effect previously observed with Taxol in each of three vehicles. Peak plasma levels in the PEG group were up to eight to ten fold higher than that observed with Taxol in suspension. These data support differential diffusion from the i.p. cavity as an explanation for the diminished antitumor results. In vitro incubation studies demonstrate that Taxol is relatively stable to plasma esterases which discounts enzymatic inactivation as an explanation of diminished antitumor response.

Additional studies will be carried out to include similar measurements of plasma Taxol in tumor bearing mice.

3. Ellipticine, NSC-71795 (Bykadi, Flora and Cradock)

Effective antitumor agents depend on the selective toxicity of the agent against

tumor cells versus normal host cells. Unfortunately, for most antitumor agents, this selectivity is minimal. Recently, considerable effort has been devoted toward techniques that promote selective drug distribution. Complexation of drug with a macromolecule can change the principal cellular uptake mechanism to pinocytosis or endocytosis. Such complexes with Adriamycin and DNA have produced selective drug distribution in rodents and in man.

Ellipticine has been shown to form complexes with native and denatured DNA. When free drug is administered intravenously, Ellipticine can produce hemolysis and considerable CNS toxicity. These factors suggest a complex of Ellipticine-DNA could offer therapeutic advantages.

Studies were initiated to investigate the feasibility of preparing and using Ellipticine-DNA complexes as antitumor agents. Initial findings showed the solubility of these complexes to be low. Complexes of Ellipticine with calf thymus or herring sperm DNA showed maximum solubilities of only about 0.15 mg/ml as free Ellipticine. A high pressure liquid chromatographic method was developed to detect small amounts of Ellipticine in biological fluids and tissues. This procedure used an ethyl acetate extraction of drug from mouse blood, plasma or tissue homogenate followed by the chromatographic separation of Ellipticine, metabolites and endogenous materials on a reverse phase column. Detection was either ultraviolet or fluorescence.

Ellipticine or Ellipticine-DNA complex was administered via lateral tail vein to BDF₁ mice at 3.0 mg/kg (as free Ellipticine). Blood and tissue samples were collected periodically and Ellipticine concentrations determined. Blood levels of Ellipticine after the administration of drug or drug-DNA complex were not markedly different. This is in contrast to the greatly elevated blood levels of Adriamycin (10-50x) seen after administration of the DNA complex of that drug. This difference in blood levels is indicative of the altered distribution. Failure of the Ellipticine-DNA complex to significantly alter blood levels may indicate the complex is reasonably unstable and dissociates rapidly after administration.

The HPLC method, however, should be useful for monitoring plasma levels of Ellipticine during clinical trials. Spiked samples of human plasma behaved similarly to that of plasma samples collected from mice administered Ellipticine intravenously. The HPLC system can also be used with a slight modification to quantitate the 9-OH metabolite. Since this metabolite does not fluoresce, a variable wavelength U.V. detector was used to quantitate 9-OH Ellipticine.

4. NSC-271674, 4-Carboxyphthalato-(1,2-diaminocyclohexane) platinum (Vishnu-vajjala and Flora)

NSC-271264 is a platinum analogue with good *in vivo* activity versus L1210 lines sensitive and resistant to Cisplatin. Formulation studies were undertaken to develop a soluble and stable pharmaceutical dosage form suitable for toxicological and clinical evaluation. Formulation studies have not been completed due to formidable analytical problems that remain under study. NSC-271674 is essentially insoluble in all common solvents, but is solubilized presumably by formation of the sodium salt of the free carboxycyclic acid group. Unfortunately, NSC-271674 rapidly degrades at neutral to alkaline pH to yield free trimellitic acid. Decomposition can be readily followed on HPLC by quantifying the generated trimellitic acid. However, we have been unable to develop a suitable HPLC or

TLC method to detect the parent compound (NSC-271674). A wide variety of normal and reverse phase stationary supports have been used without success with a large number of solvent compositions. NSC-271674 did not move in any of the HPLC or TLC systems examined to date and these studies continue.

Methods based on the generation of trimellitic acid have been used to carry out some preliminary formulation studies. Concentrations of NSC-271674, 20 mg/ml, can be readily prepared at low temperature, sterilized by filtration and freeze dried with less than 1% additional formation of trimellitic acid over four hours at 0°C. However, 26% trimellitic acid formed over the same time interval at room temperature. These studies suggest that NSC-271674 can be formulated at low temperature and used if injected within 30-60 minutes after reconstitution. A final conclusion awaits development of more definitive analytical methods.

In addition to the above mentioned studies, several small relatively straightforward projects were investigated during the past year. NSC-286193 is a new nucleoside with very interesting biological activity in the tumor panel. NSC-286193 is very water soluble and stable. The resulting formulation was a freeze dried product containing 500 mg of NSC-286193 and 200 mg of mannitol.

Studies are in progress on NSC-284356, 4,8-Ethenopyrrolo(3',4':3,4)cyclobut(1,2,-F)-isoindole-1,3,5,7(2H-6H)-tetrone, octahydro-. Adequate solubility was achieved by the formation of the disodium salt and a freeze dried dosage form has been prepared. Reconstituted solutions are quite alkaline, pH 10.0-11.4. Schedule dependency studies are in progress using the formulation.

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ANNUAL REPORT OF THE TOXICOLOGY BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 to September 30, 1981

The primary responsibility of the Toxicology Branch is to determine the preclinical toxicities of new oncolytic agents identified by the Developmental Therapeutics Program (DTP). Candidate agents which successfully pass other selection criteria within the Linear Array of the Decision Network Committee must be evaluated toxicologically prior to initiation of human Phase I trials under an Investigational New Drug Application (INDA) reviewed by the Food and Drug Administration (FDA).

The Toxicology Branch was established in March 1980 and consists of three staff members at the current time (2 professionals and 1 secretary). Toxicologic studies of new oncolytic agents under the sponsorship of the NCI are conducted by extramural laboratories operating as subcontractors to a toxicology prime contractor. The toxicology prime management contract was recompleted since the establishment of the Toxicology Branch, and is therefore operating in the first year of a three year contract period. A revised preclinical toxicology protocol has now been used in the testing of several new drugs, leading to some modifications which should facilitate passage of unprecedented numbers of chemotherapeutic agents into clinical trials.

Objectives

The objectives of the Toxicology Branch are to organize, plan, direct, manage and evaluate a collaborative contract program to: 1) determine the qualitative and quantitative toxicological impact of new and established antitumor agents and modalities on experimental animals; 2) guide the developmental research for organ specific toxicity assays for experimental drugs; 3) provide the toxicological input and assessments needed for preparation of Investigation New Drug Applications; 4) guide the development of special target organ toxicity assays needed for the development of second generation analogs of active agents through participation in the Analog Development Committee; 5) provide assistance to the Biological Response Modifiers Program in the development and conduct of special new toxicologic assays as needed; 6) provide assistance to the Pharmaceutical Resources Branch in the toxicologic assessment of experimental formulations; 7) advise the Operating Committee of the scheduling and capacity of the toxicology resources available; 8) summarize all toxicology data for the Decision Network Committee and make recommendations as to safe starting clinical doses for experimental agents; 9) conduct regular surveillance of the literature for new developments in the fields of toxicological evaluation of synthetic and natural products which may be applicable to the needs of the Developmental Therapeutics Program and Biological Response Modifiers Program; and 10) review grant applications to assist the Biochemistry and Pharmacology Grant Program of the Division of Cancer Treatment in identifying overlap between contract and grant activities and obtaining program information related to toxicological research involving therapeutic agents.

Task I Protocol Development

Protocol Development

Various factors including an uncertain supply of monkeys, diminishing resources, inordinate delays in getting new anticancer drugs to the clinics, etc., combined to require the development of a new protocol (Task I) to satisfy the FDA as adequate for the initiation of a Phase I trial of a new cytotoxic oncolytic agent. The NCI, in consultation with an FDA advisory sub-committee on toxicology guidelines for oncolytic agents, is now gaining valuable experience with the revised protocol. The new protocol requires lethality studies in mice (single and five daily doses), and toxicity studies in dogs (single and five daily doses). The dogs are observed for two months after treatment to determine: 1) the safety of the dose equivalent to 1/10 the mouse LD₅₀ on a mg/m² basis, and 2) the major organs affected by a toxic dose of the new agent.

In the spirit of cooperating fully with the FDA in adequately testing these new agents, the NCI is also determining their toxicological effects, plus those of an additional 20 oncolytic agents, in mice to determine how well the mouse qualitatively and quantitatively predicts toxicities seen in dogs and humans.

Toxicology Prime Contract

The toxicology prime management contract was recompeted this past year with Battelle Columbus Laboratories, successfully winning a new three year contract for its Toxicology Program Office in Vienna, Virginia. However, due to an unexpected influx of new agents to be evaluated toxicologically, the contract has been funded incrementally by the NCI and will have to be recompeted after only two years. There are currently four subcontracting laboratories performing Task I protocol studies under the toxicology contract, with another three to be added this year in order to increase the flexibility of the network and to expedite testing of compounds.

Two major changes are being made in the toxicology prime contractor/subcontractor relationship this year. The first involves the installation of automatic data processing equipment that allows rapid data turnaround between the contract laboratories and the toxicology prime contractor. The second is the development of a word processor-dictated final report format which will hopefully expedite report preparation at each of the subcontractors.

Oncolytics on Test (Task I)

The following agents are currently being tested for toxicities under a Task I protocol or are "anticipated" to be on test by the end of this fiscal year:

NSC No.		NSC No.	
3051	N-methylformamide	269148	"7-omen (anthracycline analog)"
71795	ellipticine	280594	tricyclic nucleoside
125973	"taxol"	284356	"a Gulf Oil compound"
141633	homoharringtonine	286193D	discreet compound
172112	spirohydantonin mustard	296934	Henkel's compound
226080	"rapamycin"	298223	"CC-1065"
241240	CBDCA (platinum analog)	301467	"a radiosensitizer"
253272D	"discreet compound"	312887	"fluoro-ara AMP"
262666	"Pettit's mustard"	328564	"a JB-11 analog"
264880	dihydro-5-azacytidine	526147	echinomycin

Tasks II and III

As mentioned earlier, under Task II studies (abbreviated or special) we are completing the retrospective intravenous LD50 mouse studies with 20 compounds as an attempt to evaluate the qualitative/quantitative predictiveness of the mouse for oncologic drug toxicities. Current Task III studies (organ specific toxicities) include the development of a neurotoxicity screen (cis-platinum, vincristine and maytansine) utilizing rats, a gastrointestinal toxicity screen (maytansine) and a dog renal toxicity screen (bleomycin, pepleomycin, and cis-platinum). All Task II and III studies are currently on hold because of financial constraints dictated by the large number of compounds being evaluated in Task I protocol studies. However, we have a continuing interest in and commitment to Task II and III studies because of their value in making program decisions about which analog of a series should be further developed.

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ANNUAL REPORT OF THE EXTRAMURAL RESEARCH AND RESOURCES BRANCH

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 to September 30, 1981

Description

The overall charge of the Extramural Research and Resources Branch is to provide direction and support for research in chemical, pharmacological and biochemical aspects of design and development of new drugs for more effective therapy of cancer. The Biochemistry and Pharmacology Grants program is concerned with studies in developmental therapeutics at the molecular, cellular, and whole body levels. The program particularly emphasizes the development and evaluation of chemotherapeutic agents used alone or in combination with other drugs and therapeutic modalities, which act specifically or selectively against malignant growth with minimal toxicity to the host. Emphasis is also placed upon studies related to the biochemical and pharmacologic aspects of mechanisms of growth and inhibition with demonstrated relevance to cancer therapy.

The Branch provides scientific guidance and program management for extramural support of research contracts, research grants, and Cancer Research Emphasis Grants in the Developmental Therapeutics Program. Branch activities cover a broad range of projects in several scientific disciplines including chemistry, biochemistry, experimental therapeutics and pharmacology. The program includes areas of drug design and synthesis, natural products research, experimental therapeutics, comparative pharmacology and mechanism of drug action studies.

The grant program encompasses a fiscal dollar level of about \$35 million and the contract program a level of between \$2-3 million. During Fiscal Year 1981, the Branch supported 358 research projects totaling 35.4 million dollars in direct costs. Careful assessment was made of program priorities to conform to fiscal constraints during Fiscal Year 1981. This analysis and the need to remain within budgetary limits has resulted in curtailment of new and competing research grants in the area of drug synthesis and natural products research. The impetus for the program to acquire novel chemical compounds and natural products to be evaluated in test systems has been slowed considerably. Effort has been made to maintain a current level of research activity in the areas of experimental therapeutics, comparative pharmacology and mechanistic drug studies. Careful surveillance of the significance of emerging data has helped to maintain emphasis on grants of high programmatic priority. Below is an analysis of the number of research projects and the amount of support in each of the Program's scientific categories for Fiscal Year 1981.

BIOCHEMISTRY AND PHARMACOLOGY GRANTS PROGRAM

FY 1981 SUMMARY BY SUB-CATEGORY
(Dollars in Thousands)

	<u>NUMBER OF GRANTS</u>	<u>TOTAL AMOUNT (Millions)</u>
SYNTHESIS & CHEMISTRY	134	11.1
NATURAL PRODUCTS	27	2.0
SCREENING & EXPERIMENTAL THERAPEUTICS	41	3.3
COMPARATIVE PHARMACOLOGY	24	2.1
OTHER PRECLINICAL ASPECTS	17	1.8
MECHANISM OF ACTION	109	10.7
PROGRAM PROJECTS	<u>6</u>	<u>4.4</u>
TOTAL	358	35.4

Significant Recent Results

Experimental Models

The capacity of tumor cells to disseminate and grow at distant anatomical sites is a crucial characteristic in malignancy, but the mechanisms involved in metastasis formation remain largely to be defined. Appropriate models of cancer cell dissemination and metastasis are indispensable requisites if progress is to be made in this area. The metastasizing capacity of spontaneous metastases of several murine tumors was investigated as an approach to test the hypothesis that metastases originate from variant subpopulations within the primary tumor.

Among transplanted murine tumors, the M5 ovarian neoplasm appears to have unique metastatic potential. The M5 ovarian neoplasm appears similar to other well characterized, highly metastatic tumors, such as the Lewis lung carcinoma. After inoculation of the tumor, spontaneous metastases were observed in liver, spleen, ovary, uterus and kidney. Lung lesions, however, were extremely rare. Spontaneous lung metastases from the M5 ovarian carcinoma were consistently observed after treatment with the antimacrophage agents silica and carrageenan. Since these compounds inhibit macrophage functions as well as natural killer (NK) activity, and the investigators have failed to find any NK activity against M5 cells, they speculate that the lack of spontaneous metastases from the M5 tumor in lungs could partly be related to tumor cell destruction by lung mononuclear phagocytes.

Murine ovarian tumors are usually non-metastasizing teratomas, a form that histologically is extremely rare in women. The M5 tumor, an anaplastic spontaneously metastasizing carcinoma, when injected i.p., grows as scattered lesions in the abdomen and results in carcinomatous ascites, as frequently observed in the human disease. Thus, the M5 ovarian tumor may represent a useful model in relation to human ovarian cancer. Studies are currently under way to characterize its responsiveness to various chemotherapeutic agents (CA 12764).

Selective Toxicity

Considerable effort has been expended in identifying biochemical characteristics unique to malignant cells which could be exploited for development of a therapeutic attack. Solid tumors are refractory to cytotoxic agents for several reasons including: a) many antineoplastic agents do not reach the poorly vascularized regions of the tumor; and b) the cellular populations in solid tumors are physiologically more heterogeneous with respect to oxygenation and proliferation than are the cellular components of hematological or particularly well-vascularized tumors. The hypoxic cells in solid tumors are refractory to effective cancer treatment. One group of investigators has described a conceptual approach for developing chemical agents which exploit the metabolic characteristics unique to cells in hypoxia, enabling selective destruction of these therapeutically resistant cells.

The therapeutic approach requires the combination of agents and/or modalities directed toward each of the cell types present in the tumor, including cycling and noncycling populations of oxygenated and hypoxic compartments. Selection of combinations of drugs should include: (a) a bioreductive alkylating agent designed to attack the hypoxic cell compartment by exploitation of the capacity of these cells to accomplish reductive reactions; mitomycin C would appear to be the most efficacious agent of this class presently available for clinical use. To maximize the differential toxicity of this agent to hypoxic cells, it should be given in relatively low doses over a relatively long period. In addition, it should be administered prior to the component(s) of the drug combination used to kill oxygenated cells, to minimize the loss of effectiveness of this agent through reoxygenation of the hypoxic cell compartment after kill of aerobic cells, a process that is capable of occurring relatively rapidly; (b) an agent such as bleomycin or a nitrosourea would seem to be a reasonable addition to such therapy to specifically attack any nonproliferating oxygenated cells present in the tumor; and (c) X-irradiation and/or an agent or mixture of agents with specificity for actively proliferating aerated cells. The drug(s) selected to attack these cellular components of the malignant tumor obviously must be capable of achieving biochemical lesions which lead to cell death (CA 02817).

Counteraction of Toxicity

A serious limitation to the clinical use of adriamycin and other anthracycline analogs is cardiac toxicity. Two cardiac effects have been reported in patients receiving adriamycin. The electrocardiographic changes are transient and occur at all dose levels regardless of schedule. Adriamycin-induced cardiomyopathy is dependent on total cumulative dose and may result in congestive heart failure.

The effects of adriamycin on the heart have been simulated in an in vitro cardiac cell culture system. At high adriamycin doses, cessation of beating was rapid and structural changes consistent with the in vivo cardiomyopathic picture were observed. At low adriamycin doses arrhythmias were produced in the absence of ultrastructural changes. The incidence and severity of the arrhythmias were demonstrated to be dose dependent. Continued treatment of cultures at low dose levels for sustained periods of time resulted in a striking loss of muscle fiber without concomitant vacuolization and nucleolar fragmentation.

The parallel between the effects of adriamycin on in vitro cardiac cell structure and function with those seen in vivo suggests that this simple system may have value in studies directed towards the mechanism of adriamycin induced cardiac toxicity and in the screening of anthracycline analogs for their potential effects on the heart (CA 24771).

Gene Manipulation and Drug Resistance

Newly emerging techniques of molecular biology involving artificially recombinant DNA are uncovering numerous examples of the phenomenon of an organism's complete set of genes becoming larger in terms of both the amount of the genetic material DNA and the number of genes. This evolutionary phenomenon is thought to result mainly from gene amplification, the process whereby a small part of the genome, representing one or more genes, is duplicated locally within a chromosome.

Researchers are examining the mechanism whereby cultured mouse and hamster cells become resistant to the drug methotrexate. Methotrexate has long been a standard drug for the treatment of various forms of cancer, and it has been established that these cancers can become resistant to the drug. The cells that are resistant to the methotrexate contain as many as 200 times the normal number of genes for the enzyme dihydrofolate reductase. That enzyme is the target of the anti-tumor drug. New preliminary evidence indicates that the tumors of patients treated with methotrexate can become resistant by making extra copies of the gene. The extra genes seem to be situated first on small, two-part, self-replicating units of DNA, which are called "double minutes." The extra genes can become more permanently incorporated into the cell's genetic information by attaching to one of the cell's chromosomes. Gene amplification is likely to be a common mechanism for the development of drug resistance.

The investigators plan to continue studies to determine the mechanisms of generation of drug resistance in order to produce drugs or to employ splicing and recombinant DNA techniques in an effort to modify or prevent the emergence of resistance to drugs (CA 16318).

Anticancer 'Prodrugs'

A persistent problem in cancer chemotherapy is that the concentrations needed to kill tumor cells are close to those that produce severe toxicity to the host. One often-proposed approach to overcoming this problem has been to design anti-cancer "prodrugs" which are inactive until locally activated by some tumor-associated enzyme.

Many types of malignant cells and human tumors display increased concentrations of the protease plasminogen activator that converts plasminogen to the highly active protease, plasmin. Plasmin rapidly cleaves various low molecular weight compounds coupled to appropriate peptide specifiers.

One research group showed that two anticancer drugs having widely different mechanisms of action, an antimetabolite and an alkylating agent, can be linked to peptide specifiers to create inactive "prodrugs" that are substrates for, and can be activated by, plasmin. It is felt that plasmin-activated "prodrugs" of the type described provide a reasonable prospect for improved therapeutic effectiveness in cancer chemotherapy.

Drugs with a wide variety of pharmacological properties and modes of action can be converted into plasmin-activated "prodrugs." Although it is far more difficult to demonstrate an increase in drug selectivity in vivo than in vitro because factors of drug distribution, clearance, and activation at extratumor sites become important, it is felt that the improved selectivity demonstrated at the cellular level in vitro augurs well for the possibility of demonstrating improved selectivity in vivo (CA 29837).

Cell Cycle: Drug Effects

Most solid tumors are composed of populations of dividing and nondividing cells. At least part of the tumor's response to chemotherapy will depend upon the differential sensitivities expressed by the cell in these two populations. Research is continuing on the characterizations of drug effects on cell kinetics and survival rates.

The anticancer drugs in current use produce some form of cytotoxicity to tumor cells as well as to the normal cells in the body. Perhaps the greatest toxic side effects are observed in cell renewal systems such as skin, hematopoietic system and small intestine.

Because of a better knowledge of drug effects on the cell cycle, it may now be possible to manipulate a patient's tumor kinetics in a way that would optimize treatment scheduling. An example of a kinetics directed protocol is the combination of vincristine and bleomycin. Vincristine blocks cells in mitosis and bleomycin kills cells best in mitosis. Therefore, if vincristine were to be administered first to enrich the fraction of cells in mitosis and if bleomycin was given some time later, when the mitotic index was increased several fold, then the effects of bleomycin would be greatly enhanced. This schedule is being used currently as a very effective chemotherapy protocol in the treatment of solid tumors. Other examples can be given, but it should be clear that the characterizations of drug effects on survival and cell kinetics are valuable parameters to consider when planning chemotherapy protocols. This rationale, coupled with a better knowledge of the tumor population kinetics, should make it possible to design more effective tumor treatments (CA 15397).

C-Nucleoside Synthesis

A relatively new group of naturally-occurring nucleosides, the C-nucleosides are antibiotics which exhibit anticancer activity. Chemical syntheses in the area of C-nucleosides have already produced several target compounds which exhibit exceptional activity in vitro and in vivo. This area of research offers promises

to become a fertile source of potential anticancer agents. New leads obtained from biological evaluation of these compounds combined with the synthetic methodology now being developed will offer the possibility to design new C-nucleosides with enhanced selectivity and improved chemotherapeutic indices.

In one study the synthesis of the adenosine analog of the 9-deazapurine nucleosides of Class V was completed by the synthetic sequence. In vitro studies demonstrated that 9-deazaadenosine possesses exceptional growth inhibitory activity in several systems.

Studies in mice showed also that this compound had significant chemotherapeutic activity against L-1210 leukemias. It is noteworthy that deoxycoformycin (a potent inhibitor of adenosine deaminase) does not enhance the effect of 9-deazaadenosine which would imply that the latter is not appreciably deaminated in vivo (CA 24634).

Emphases and Projections

Techniques for insertion of new genes through recombinant DNA is an exciting area of emphasis. Future plans include development of more efficient techniques for insertion of new genetic information into cells, and approaches for applying this technology to develop new and unique drugs for treatment of cancer patients through gene replacement therapy.

High priority is accorded to development of new multi-drug regimens through close pharmacokinetic and biochemical monitoring of the effects of multi-drug regimens on neoplastic and normal tissues. Knowledge of the underlying pharmacological and biochemical rationales which produce therapeutic synergism will make the choice of drug combinations, as well as their optimal scheduling, a more predictable and rational process. Research in this area will lead to improved responses by the eventual tailoring of multi-drug chemotherapy to fit the individual cancer patient.

Emphasis is being placed on improving therapeutic ratio of most clinically effective antimetabolites. Modulation of antimetabolite activity constitutes an important approach to improving the therapeutic ratio of these drugs. By evaluating combinations of drugs and/or salvage pathway metabolites for their ability to increase selectivity, advances will be made that may result in substantially better treatment programs for cancer patients.

Another area of emphasis in this program is research projects involving mechanisms of membrane transport and its role on drug effectiveness. Transport of antifolates as it relates to the free intracellular antifolate level is clearly a critical element in cytotoxicity and selectivity. New developments in the area of methotrexate transport will be further explored to reveal information on the role of transport in other drug-cell interactions and to further basic investigation that might be applied to enhance chemotherapeutic effectiveness.

There is increasing awareness that the use of known and established clinical drugs can be manipulated to induce more effective and longer responses in cancer patients. Toxicity of drugs such as methotrexate and adriamycin in higher doses can be modified by manipulation of dosage regimens or by employment of rescue factors with ultimate protection to patients from severe

toxic side effects. Use of high-dose therapy with appropriate counteractive rescue agents appears to be especially promising for patients with metastatic solid tumors.

Development of analogs of cancer drugs is a promising area of research. There is an urgent need to develop analogs of cancer drugs which will be at least as effective as the parent drug, but which will produce fewer side effects. By developing analogs of Adriamycin, a particularly effective cancer cell killer, it is possible to reduce or to eliminate the cardiac toxicity associated with the parent drug. A part of the search for understanding of the biochemical and pharmacological parameters involved in effective analogs is conducted through development of better new drugs, new initiatives for use with in vitro test systems, and advanced tests for measuring the sensitivity to anticancer drugs of specific human tumor cells. By correlating in vitro and in vivo test data, the most effective drug may be selected for treatment of individual cancer patients.

Other Program Activities

Conference Support: The Extramural Research and Resources Branch provided partial support for two conferences during 1981. The fourth Gordon Conference in the series on Chemotherapy of Experimental and Clinical Cancer was held at the Plymouth State College, Plymouth, New Hampshire on July 27-31, 1981. The purpose of the Conference was to emphasize areas of cancer chemotherapy that appear promising at the present, that represent relatively new areas, and that represent areas likely to have a major impact on clinical therapeutics. The proposed program included sessions on brain tumors, platinum compounds as anti-tumor agents, cell differentiation and chemotherapy, long-term effects of chemotherapy and biological response modifiers, along with other topics. The conference has become a forum for review of current data and testing of new concepts and their implications by basic scientists and clinicians in the research areas of chemotherapy.

The Symposium on the Design of New Antineoplastic Agents was held at the Amherst Campus of the State University of New York at Buffalo, New York, on May 18-20, 1981. The purpose of this symposium was to stimulate new ideas and endeavors aimed at synthesis of more effective anticancer agents. The symposium has been well-attended for many years. This year outstanding speakers discussed issues aimed directly on new rationales and biochemical and pharmacological considerations regulating the design of anticancer agents.

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ANNUAL REPORT OF THE LABORATORY OF CHEMICAL PHARMACOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

OCTOBER 1, 1980 to September 30, 1981

Efforts in the Laboratory of Chemical Pharmacology, DTP, DCT are concerned with conducting research, teaching and training, contract administration, and providing administrative and scientific support to the Developmental Therapeutics Program and the Division of Cancer Treatment.

The study of new as well as established antitumor agents is the primary research activity of the Laboratory of Chemical Pharmacology. These studies are chiefly concerned with elucidating the pharmacological properties of such agents, and include an evaluation of their disposition and metabolism, their mechanism of action, the mechanisms by which tumor cells become resistant to them, and their potential adverse effects. Studies are conducted not only in laboratory animals, but also in humans, and the test compounds include antitumor agents as well as other foreign compounds used as models for defining specific processes or mechanisms. Of special concern in the disposition studies of new antitumor agents is the tissue distribution of the compound, including its ability to cross the blood-brain barrier; in addition, the lymphatic absorption of the compound, its biotransformation and its rate of elimination are evaluated in parallel with attempts to develop a model for its pharmacokinetic behavior. These studies require in many instances the development of new analytical methodologies to facilitate pharmacologic studies of antineoplastic agents whose disposition and metabolic fate is not fully understood.

Other studies in the Laboratory of Chemical Pharmacology are concerned with the development of novel approaches and combined modality regimens for tumor therapy, the rational design of new antitumor agents, evaluation of chemotherapeutic agents in various model tumor systems, and characterization of the transport properties of the blood-brain-CSF system. Efforts are also underway to characterize the toxicity of new antitumor agents, and to develop methods of reversing their toxicity.

Studies on the pharmacology and metabolism of several antitumor agents and radiosensitizers, including misonidazole, desmethylmisonidazole and m-AMSA, have been carried out during the past year. Both misonidazole and its metabolite, desmethylmisonidazole, are effective hypoxic cell sensitizers; clinical studies with misonidazole have shown that its dose limiting toxicity is neurotoxicity, but the relationship between neurotoxicity and the metabolism of misonidazole is not clear. In a study of the O-demethylation of misonidazole to desmethylmisonidazole by rat liver microsomes it was found that a system comprised of microsomes, oxygen and NADPH was optimal. Metabolism was inhibited by CO, N₂ and SKF-525A; phenobarbital-induced microsomes produced a significant increase in the V_{max} of the reaction. The results indicate that the O-demethylation of misonidazole is mediated by a cytochrome P450 mixed function oxidase.

Rat liver microsomes were also used to study the mechanism of misonidazole toxicity. In particular, enzymatic conversion of the drug to metabolites sufficiently reactive to bind to tissue macromolecules was evaluated, since it

distribution half time of 9 min + 6 min SD and a terminal plasma elimination half life of 6.1 hrs + .5 hr SD; 50% of the total dose was excreted in the urine. The apparent volume of distribution averaged 766 ml/kg + 100 ml/kg. These data indicate that, as observed in animals, desmethylmisonidazole has a shorter elimination half time and higher renal excretion than does misonidazole and may offer pharmacological advantages over the parent compound as a radiosensitizer.

Studies on the pharmacology and mechanism of action of m-AMSA have continued during the past year. Using rat liver microsomes, it was found that the metabolism of m-AMSA is optimal in a system comprised of microsomes, cytosol, oxygen and an NADPH generating system. Metabolism was decreased by omitting the NADPH generating system, microsomes or cytosol and was inhibited by CO and N₂. Inhibitors of hepatic microsomal mediated drug oxidation (β -naphthoflavone, metyrapone and SKF-525A) reduced metabolism, whereas phenobarbital or 3-methyl cholanthrene pretreatment resulted in a marked stimulation. HPLC analysis revealed that rat liver microsomes incubated with m-AMSA form two products: N'-methanesulfonyl-N-(9-acridinyl)-3'-methoxy-2', 5'-cyclohexadiene-1', 4'-diimine (m-AQDI) and 3-methoxy-4'-(9-acridinylamino)-2', 5'-cyclohexadien-1'-one (m-AQI). Since m-AMSA can be formed via an N-hydroxylated intermediate, the metabolism of m-AMSA is postulated to proceed via this route, resulting in bioactivation to a highly reactive species. An *in vitro* cell colony assay in soft agar utilizing L1210 cells was established to determine the relative cytotoxicities of m-AMSA, m-AQDI and m-AQI. L1210 cells exposed to m-AMSA showed a degree of cell-kill that was dependent on concentration and length of exposure up to 4 hours. m-AQDI and m-AQI are approximately 100-fold more toxic than m-AMSA, and unlike m-AMSA, toxicity is not time-dependent. The principal *in vivo* biliary metabolite of m-AMSA, m-AMSA-5-glutathione, was not toxic. Derivatives of m-AMSA were synthesized in which the nitrogen of the anilino ring was substituted to form a tertiary amine. These derivatives were non-toxic, supporting the hypothesis that N-hydroxylation is the initial reaction in the metabolism of m-AMSA. These results indicate that m-AMSA is bioactivated with time *in vivo* to a highly reactive species capable of reacting with critical macromolecules causing cell death, or with molecules such as glutathione resulting in detoxification.

o-AMSA, an analogue of m-AMSA, is devoid of antitumor activity and has reduced *in vitro* cytotoxic activity. Previous biochemical and biophysical studies have failed to distinguish properties of m-AMSA and o-AMSA that would account for their difference in activity. However, the oxidation products of o-AMSA (o-AQDI and o-AQI), are as cytotoxic as m-AQDI and m-AQI. If o-AMSA is inactive because it is not bioactivated, then the activity of m-AMSA would be related to its intracellular bioactivation. o-AMSA was found to be metabolized in a system comprised of microsomes, oxygen, and a NADPH generating system. The need for cytosol was less critical than in m-AMSA metabolism. At equimolar concentrations, o-AMSA was metabolized at a significantly greater rate than m-AMSA. o-AMSA was found to competitively inhibit the metabolism of m-AMSA, while m-AMSA appeared to non-competitively inhibit the metabolism of o-AMSA. In addition, the main biliary metabolite of o-AMSA in rats demonstrated an R_f differing from that of m-AMSA as determined by TLC. These preliminary studies indicate that o-AMSA may be metabolized by a different microsomal pathway than m-AMSA and that this could account for the difference in antitumor activity. It has not been possible to detect significant binding of m-AMSA to the DNA of L1210 cells; however, it has been found that m-AMSA, m-AQDI and m-AQI cause DNA strand breaks. Similar studies on the o-AMSA series revealed that, while o-AMSA

is known that various aromatic nitro compounds require metabolic reduction of the nitro group to a reactive species for toxicity.

[¹⁴C]-misonidazole was metabolized by rat liver microsomes and purified NADPH-cytochrome C reductase anaerobically to a reactive intermediate that covalently binds to tissue macromolecules. Air strongly inhibited the binding whereas carbon monoxide had no effect, indicating that misonidazole is activated via reduction and not by cytochrome P450 dependent oxidation. Both systems showed an absolute requirement for NADPH and were stimulated by flavine (FAD) and paraquat. At a single substrate concentration nitrofurantoin, nitrofurazone and desmethylmisonidazole inhibited the covalent binding of misonidazole to protein by 47, 26, and 38%, respectively. Glutathione reduced the binding of misonidazole to microsomal protein below the level observed for boiled microsomes while ascorbic acid had no effect. Compared to nitrofurantoin and paraquat, misonidazole was a poor stimulator of superoxide production as measured by adrenochrome formation.

The isolated perfused rat liver was employed to examine the dose-dependent clearance of misonidazole and to gain an insight into the mechanism(s) involved in the clearance of both misonidazole and desmethylmisonidazole by that organ. The simplest model which accurately described the desmethylmisonidazole clearance from the perfusate consists of a saturable elimination pathway ($V_{max_3}=32$ nmol/min, $K_{m_3}=11$ μ M) in parallel with a first order pathway ($Cl_5=.21$ ml/min). A similar model was constructed for misonidazole ($V_{max_2}=110$ nmol/min, $K_{m_2}=10$ μ M, $Cl=.36$ ml/min) but required an additional saturable pathway ($V_{max_1}=226$ nmol/min, $K_{m_1}=1850$ μ M) to characterize the generation of desmethylmisonidazole, as suggested by *in vitro* rat liver microsomal studies. A good correlation was demonstrated between desmethylmisonidazole perfusate concentrations (generated during the course of misonidazole perfusions) and simulations based on the misonidazole model which included the microsomal data. The misonidazole and desmethylmisonidazole models demonstrate that the relative contributions of the different pathways for misonidazole and desmethylmisonidazole elimination are strongly concentration-dependent and that the misonidazole $\rightarrow$ desmethylmisonidazole pathway is a minor route. A qualitative similarity in saturation kinetics was observed in the disappearance curves for both misonidazole and desmethylmisonidazole from the liver perfusate. When misonidazole was combined with an excess of desmethylmisonidazole, a marked decrease in the clearance rate of misonidazole from the liver perfusate was noted. This observation suggests that misonidazole and desmethylmisonidazole are metabolized along similar routes.

As noted above, clinical studies have indicated that the dose limiting toxicity associated with misonidazole is neurotoxicity. Previous clinical studies at NCI with intravenous administration of misonidazole have shown a strong correlation between the extent of neurotoxicity and the area under the plasma misonidazole concentration-time curve. This is in agreement with results reported by other investigators following oral administration of misonidazole. Desmethylmisonidazole yields X-ray dose enhancement ratios in animals similar to misonidazole and may possess pharmacological advantages over the parent compound. A comparison of the pharmacokinetics of misonidazole and desmethylmisonidazole in animals showed that the latter compound has a reduced area under the plasma concentration-time curve, a shorter plasma elimination half life, and increased urinary excretion. Based on these observations, clinical trials of desmethylmisonidazole have been initiated. To date, two patients at NCI have received doses of 1 g/m² and 2 g/m². The results of the analysis showed an initial mean

caused no DNA breaks, the activated intermediate o-AQI was capable of inducing DNA breaks in L1210.

Although m-AMSA has routinely been administered to patients by the intravenous route, it has been given orally as well. Plasma concentrations of m-AMSA were much lower after oral than IV dosing, possibly due to poor gut absorption of drug, its rapid clearance from the portal system, or both. Studies to evaluate these possibilities showed that, in rats, m-AMSA was readily absorbed from the gut. Therefore, the low plasma levels noted after oral dosing must be caused by rapid removal of drug by the liver. This rapid hepatic extraction gives rise to the possibility of enterohepatic recirculation of parent drug or of metabolite. However, parabiosis experiments in rats given ^{14}C -m-AMSA showed that only 9.3% of radioactivity in bile was reabsorbed from the gut. These results indicate that some enterohepatic recirculation of m-AMSA and its metabolites does occur, but the proportion of drug recirculated is small and probably clinically insignificant.

m-AMSA has been used with considerable clinical success against the leukemias, with complete remissions reaching 30% in previously treated patients. Because of its anti-leukemic activity, m-AMSA is being considered as a third-line drug against meningeal leukemia which is refractory to standard therapy. Previous work has shown that m-AMSA crosses the blood-brain barrier with difficulty. Intraventricular administration of 50 μg m-AMSA to monkeys showed a probable biphasic clearance from the CSF with peak concentrations of $2.4 \times 10^{-5}\text{M}$. When the administered dose was increased to 500 μg , the peak concentration was increased ten-fold to $2.3 \times 10^{-4}\text{M}$. At neither dose, 50 μg or 500 μg , did the monkeys exhibit significant neurotoxicity. m-AMSA was also administered (500 μg in 0.8 ml of steroid suspending vehicle) by lumbar puncture and samplings were made from the 4th ventricle through an Ommaya reservoir. These studies are not yet completed but, so far, drug has not been detected in the ventricular fluid. If the drug can not enter ventricular fluid following lumbar puncture, its use in meningeal disease will be restricted to patients with Ommaya reservoirs in place.

A variety of studies on the distribution of drugs between blood, brain and the CSF have been performed during the past year. The technique of qualitative autoradiography (QAR), described in previous Annual Reports, has been further refined and applied to physiologic studies such as measurement of cerebral or other tissue blood flow, local glucose utilization, protein turnover, and measurements of capillary and cellular permeability. QAR has also been used to study the distribution of ^{14}C -methotrexate, ^{14}C -thymidine and ^{14}C -misonidazole in normal and tumor-bearing animals. Two new variations of the QAR methodology have been devised. One of these variations makes it possible to do simple solvent extractions on frozen and dried tissue sections and determine the amounts of soluble and bound carbon-14 or other radioactive nuclide present in the tissue; this has been employed in studies of the distribution of ^{14}C -leucine and ^{14}C -thymidine. The other variation makes it possible to histologically prepare for microscopic examination the same tissue section which was used for QAR (in the past the histological data was gained from the fixing and staining of the tissue section adjacent to the one taken for QAR); this procedure makes for more precise correlations between the morphology and physiology of the tissue section.

Pathophysiological studies have been performed on 5 brain tumor models: the Walker metastatic carcinoma, the ENU-induced primary brain tumor, and brain tumors induced by the direct implantation of avian sarcoma virus (ASV), RG-2

astrocytoma cells or astrocytoma RT-G cells into the brain of rats. Of these five brain tumor models, the virally-induced glioma (ASV) has been studied most extensively this year. In a double label QAR study, tissue blood flow (F) was measured with ^{131}I -iodoantipyrine (IAP) and the blood-to-tissue transfer constant (K_1) obtained with ^{14}C -AIB, and marked regional variations in F and K_1 as well as histological appearance were found. In the large tumors (2-10 mm diameter), the K_1 's of AIB were increased 20-150 fold in the center, 6-200 fold at the tumor edge, and several fold over normal brain in the tissue surrounding the tumor; moreover, F was consistently and dramatically diminished in the center of these tumors ($< .06 \text{ ml/g} \cdot \text{min}$), slightly decreased to normal at the edge ($.25\text{--}1.0 \text{ ml/g} \cdot \text{min}$), and definitely diminished in brain tissue around the lesion ($.06\text{--}.6 \text{ ml/g} \cdot \text{min}$). In the small tumors ($< 2 \text{ mm}$ diameter), K_1 was modestly increased throughout (2-20 fold) and was normal in the surrounding neural tissue, whereas F was slightly decreased or normal within the tumor and the surrounding brain. These alterations in K_1 and F were correlated more with tumor size than with tumor histology.

Simultaneous measurements of local glucose utilization rate (R) and F were made in a second group of ASV rats using double-label QAR of ^{14}C -2-deoxyglucose (2-DG), and ^{131}I -IAP. The tumors which were found and studied were relatively small ($< 3 \text{ mm}$ in diameter), with one exception, but quite variable in histological appearance. The same relationship between tumor size and F as previously presented for this model system was found. Although differences in R were observed within each tumor, most of them showed a similar range of rates ($30\text{--}50 \mu\text{M glucose}/100\text{g} \cdot \text{min}$) and patterns of R; however R values greater than $100 \mu\text{M}/100\text{g} \cdot \text{min}$ were found in the one large tumor, a polymorphic glioma. The local glucose utilization rate did not seem to correlate with tumor size, histology, cytology, or cellular density in the ASV model.

An investigation of the distribution of ^{14}C -misonidazole (MISO) in rats with RT-9 gliomas in their flanks was undertaken using QAR and HPLC. A very heterogeneous distribution of MISO-derived radioactivity was observed at the various sampling times. Appreciable amounts of radioactivity were found only around the rim of the tumor 5-10 minutes after bolus intravenous injection. In experiments where constant blood ^{14}C -activities were maintained by the continuous infusion of labelled MISO, the same large discrepancy in concentrations between tumor rim and center was seen. At all tissue sampling times, the disposition of the infused radioactivity was not uniform within the centers of the tumors; there were islands of cells therein with considerably higher amounts of activity than adjacent necrotic areas. Double level QAR experiments with ^{14}C -MISO and ^{131}I -IAP showed that the blood flow (IAP) and MISO distribution patterns were very similar and that delivery of MISO in this tumor system is greatly limited by blood flow. HPLC analysis also indicated that less than 40% of the ^{14}C -activity was associated with the parent drug after 4 hours of MISO infusion. Metabolism, therefore, may also compromise the disposition of MISO within this tumor model.

The possibility of "opening" the blood-brain barrier (BBB) by the inhalation of 20% CO_2 for 2 hours or more was investigated by multiple isotope detection. This treatment had no discernable effect on the blood-brain K_1 's of two substances, the moderately permeable ion, ^{42}K , and the slightly permeable amino acid, AIB; however the transfer across the BBB of DTPA, a virtually impermeable compound, was increased 5-20 fold by the exposure of high blood levels of CO_2 . This peculiar change in BBB permeability suggests that CO_2 inhalation may enhance the exchangeability of very impermeable drugs between blood and brain.

Another area of research involves studies on the biochemical, toxicological and antineoplastic properties of a series of sulfhydryl derivatives of cyclophosphamide and polymeric analogs of these compounds. The compounds were linked to DIVEMA, a negatively charged polymer whose immunestimulating characteristics served as the basis for linking it to immunosuppressive antitumor agents. The in vitro cytotoxicity and DNA damaging effects of one of the sulfhydryl derivatives of cyclophosphamide, alone or linked to DIVEMA, were compared. The sulfhydryl derivative consistently produced twice the cell kill (soft agar colony formation of L1210) and twice the interstrand DNA crosslinks (alkaline elution assay) as did an equivalent amount of sulfhydryl derivative linked to DIVEMA, indicating that the attachment of a low molecular weight antineoplastic agent to a highly negatively charged polymer can substantially alter the interaction of the agent with tissues. The biological effects of synthetic polymers with a variety of charge densities are being explored. Polyethyleneimine N-2 carbonyl-ethyl-ethyleneimine copolymers with various degrees of reduced negative charges have been attached to the sulfhydryl cyclophosphamide derivatives. Results thus far indicate that these compounds are less toxic and more active against L1210 in vivo than their DIVEMA analogues.

The ability of liposomal encapsulation of antitumor agents to alter their disposition, lymphatic uptake, and therapeutic effects against experimental lymph node metastasis has also been evaluated during the past year. Adriamycin was entrapped in liposomes and its disposition compared with that of free adriamycin at intervals after ip dosing to rats. Liposomal encapsulation increased the concentration of drug equivalents in liver, spleen and diaphragm as well as in those lymph nodes through which lymph draining the peritoneal cavity passes. After iv dosing a similar increase in the uptake of drug equivalents by liver and spleen was noted but not by diaphragm or lymph nodes. Liposomal encapsulation also altered the peritoneal absorption, tissue distribution, metabolism and excretion of ara-C. Liposome entrapment of ara-C reduced the rates at which the drug was absorbed from the peritoneal cavity and excreted in urine while enhancing lymphatic uptake of the drug by more than 10-fold. Radioactivity in plasma and most tissues achieved higher concentrations and persisted for longer periods in rats given liposome entrapped ara-C than in rats receiving the free drug. Most striking was the localization of ^{14}C -activity in renal and thoracic lymph nodes of rats given liposome entrapped ara-C, with 300-1000 fold higher levels present at 4, 12 and 24 hr post treatment than in corresponding lymph nodes of rats receiving the free drug. The metabolic conversion of ara-C to uracil- β -D-arabino-furanoside was reduced by ~3 fold following liposome entrapment of the drug. The enhanced lymphatic uptake and the localization and persistence of adriamycin and ara-C in lymph nodes resulting from liposome entrapment of the drugs may be of benefit in treating tumors that metastasize via lymphatic pathways.

In order to assess the therapeutic effects of liposome-encapsulated antitumor agents against lymphogeneous metastases, it has been necessary to establish a model for lymph node metastasis which is reproducible and quantitative. Two rat tumors, Walker 256 carcinoma and 13762 mammary adenocarcinoma, have been developed for this purpose. Tumor cell suspensions are inoculated subcutaneously in the hind leg of rats, and at various intervals after inoculation the tumor-bearing leg is amputated. For both tumors, metastasis takes place by lymphatics to regional lymph nodes and by hematogenous spread to lungs. For the mammary adenocarcinoma 13762, the time interval between inoculation and amputation has been found to be a critical determinant in the extent of lymph node involvement with tumor, as determined by serial measurement of lymph node weight or volume,

and in the survival time of the animal. Amputation prior to 5 days after tumor inoculation reduces the incidence of blood-born lung metastases but also renders the development of lymph node metastasis less reproducible. Amputation on day 9 or 10 after tumor inoculation results in regional lymph node metastases in 100% of the animals at risk, and the weight of the lymph node has been found to reflect the degree of tumor involvement; however, the animals ultimately die of lung metastases.

The effect of liposomal entrapment of melphalan (MPL) on its tissue distribution and activity against lymph node metastasis of the 13672 adenocarcinoma was evaluated in rats. Neutral small liposomes containing MPL and the fluorescent marker carboxyfluorescein (CF) were prepared by sonication using phosphatidylcholine (PC) and cholesterol, along with tracer amounts of ^{14}C -MPL and ^3H -PC. Free ^{14}C -MPL injected sc into the thigh of rats was rapidly cleared from the injection site, with only 0.6% of the ^{14}C activity remaining after 2 hours. The concentration of MPL in the ipsilateral inguinal lymph nodes showed a transient increase over that in contralateral nodes at 0.5 hour, although by 1 hour MPL concentrations were similar in ipsi- and contra-lateral nodes. After sc injection of ^{14}C -MPL/ ^3H -PC-liposomes, about 4% of the ^{14}C activity was present at the injection site at 2 hour post treatment. The concentration of PC equivalents in ipsilateral lymph nodes was at least 50 times higher than in plasma, lung or contralateral lymph nodes at all time intervals examined. Similarly, liposomal entrapment of MPL enhanced its uptake by ipsilateral lymph nodes; this increase in lymph node MPL concentration was sustained for at least 24 hours after dosing, at which time ipsilateral nodes contained 20-fold and 10-fold higher MPL levels than were present in plasma and contralateral nodes, respectively. Rats bearing the 13672 adenocarcinoma were given a single sc injection of free or liposome-entrapped MPL 3 days after surgical resection of the primary tumor. Liposome-entrapped MPL produced a greater reduction in the weight of lymph node metastases than did free MPL. Thus, when MPL was entrapped in liposomes, a dose of 0.125 mg/kg reduced the weight of lymph node metastases to about 50% of controls. A dose of 0.5-1.0 mg/kg was required to exert an equivalent effect on lymph node tumor growth when free MPL was administered. These results suggest that the interstitial injection of liposomes containing MPL may be useful for the postoperative treatment of lymph node metastases.

The toxic and therapeutic effects of thymidine, alone and in combination with ara-C, has been examined. Thymidine alone suppresses L1210 growth in vitro if adequate concentrations and exposure times are maintained. The cytostatic effect (growth inhibition assay) of thymidine was compared with its cytotoxic effect (inhibition of colony formation) at various concentrations and exposure times. Cytostasis was achieved at concentrations of thymidine of 0.1 mM, but if thymidine is removed by washing, concentrations approaching 1 mM are needed to sustain cytostasis for 120 hours. Pharmacokinetic studies of thymidine in mice indicated that such conditions of concentration and exposure time could be achieved with multiple injections or constant intraperitoneal infusions with tolerable toxicity. Cytotoxic effects in vitro indicated that a three log cell kill could be obtained at in vivo pharmacokinetically achievable concentrations and exposure times. However extensive in vivo trials indicated no measurable cytostatic or cytotoxic effect of thymidine in L1210 leukemia. This ineffectiveness could not be explained on a pharmacokinetic basis (e.g., lack of sufficient thymidine concentration and exposure time). It is known that thymidine exerts its cytotoxic effect through thymidine-triphosphate's (TTP) inhibitory effect on the enzyme ribonucleotide reductase. This inhibition leads to depletion of dCTP,

a necessary substrate for DNA synthesis. Thymidine cytotoxicity can be reversed if the salvage metabolite, deoxycytidine, is supplied in sufficient quantity. The plasma deoxycytidine concentration of either normal or L1210-bearing mice prior to thymidine treatment was not sufficiently high to explain the lack of effect of thymidine on the growth of L1210 *in vivo*. However following thymidine treatment of mice both cytidine and deoxycytidine in plasma and spleen rose to concentrations that were capable of reversing thymidine cytostasis or cytotoxicity *in vivo*. The mechanism by which thymidine causes a rise in deoxycytidine in plasma is being investigated.

Resistance of tumors to ara-C is very likely due to an imbalance of the deaminases and kinases that inactivate and activate ara-C. The metabolic product of thymidine, TTP, is a potent inhibitor of cytidine deaminase as well as an inhibitor of pyrimidine kinases and ribonucleotide reductases. The possibility that this selective action on certain enzymes may extend to chemotherapeutic selectivity against tumors versus normal tissues when ara-C and thymidine are used in combination is under study. Using L1210, a tumor which is sensitive to ara-C, it was found that thymidine shifts the dose response curve for ara-C toxicity far to the left, i.e. much less ara-C has to be given for equal toxicity to mice when given with thymidine than when given alone. Thymidine given simultaneously with ara-C results in greater toxicity than when there is a delay between thymidine administration and ara-C. In addition, better therapeutic results were obtained when there was delay between administration of ara-C and of thymidine than when the drugs were given simultaneously. However ara-C given alone at equitoxic doses to the above combination was superior in therapeutic effects against L1210. The timing of this combination is obviously very important and an investigation of intracellular nucleotides may give a better understanding of the biochemical events that are involved in this phenomenon.

Studies were continued on the biologic importance of circulating uridine and the relative importance of the *de novo* and salvage pathways for uridine nucleotide biosynthesis. A study of the bioregulation of circulating uridine in rats revealed that ^{14}C -uridine was rapidly cleared from the circulation after iv dosing, with a half-life of 1-4 minutes. When labelled uridine was injected along with 1000-10,000 nmol of unlabelled uridine (normal rat plasma uridine levels are 1.5-4.7 nmol/ml), plasma uridines levels rapidly returned to normal, and the half-life of ^{14}C -uridine remained in the range of minutes. The role of the liver as source and/or regulatory mechanism of circulating uridine concentrations was investigated using an isolated perfused rat liver system developed over the past year. The isolated rat liver excreted uridine into a circulating perfusion medium achieving concentrations similar to those found in rat plasma (1.4 ± 0.6 mM), and the rate of excretion was altered by changes in the circulating concentrations of uridine. A 10 fold decrease in output was observed when the excreted uridine was allowed to increase until an equilibrium was reached at approximately 1 μM . At this steady state concentration, a single radioactive uridine spike is rapidly lost from the perfusate by an apparent first order process, with a half life of 7.4 min. This elimination is apparently due mainly to the rapid distribution of the radioactive uridine into the uracil nucleotide pool of the perfused liver since the specific activity of the circulating uridine was reduced by a factor equivalent to the concentration of total hepatic uracil nucleotides. There was also catabolism of the circulating uridine, as evidenced by chromatographic analysis of the perfusate. Uridine is stored in the liver as the nucleotide, not the nucleoside, and therefore the equilibrium concentration of approximately 1 μM achieved in the plasma reflects a balance between uridine

kinase and 5'nucleotidase activity.

Cells that contain uridine/cytidine kinase have the ability to utilize extracellular uridine in order to circumvent inhibition of the de novo pyrimidine biosynthetic pathway. The ability of uridine at normal serum levels for humans, rats, and mice (2-12 μ M) to reverse the cell growth inhibitory effects of PALA, an inhibitor of de novo pyrimidine biosynthesis, was determined in tissue culture. Since both L1210 cells and Lewis lung carcinoma cells rapidly deplete uridine from cell culture media, a method for infusing uridine was devised in order to maintain the media uridine at constant levels. Extracellular uridine at normal serum levels was found capable of markedly reducing the growth inhibitory effects of PALA on both cell lines. Thus serum uridine may reduce the antitumor effectiveness of inhibitors of the de novo pyrimidine biosynthetic pathway in vivo. Three such inhibitors were studied to determine their effects on circulating uridine concentrations in BDF₁ mice. Pyrazofurin and 6-azauridine had no significant effects on serum uridine levels, whereas PALA reduced serum uridine levels by 55%. This reduction could contribute to the antitumor effectiveness of PALA by limiting the rescue of cells possessing a salvage pathway. Although serum uridine levels in patients treated with PALA were also found to decrease from predose serum levels, they generally remained in the range of serum levels for normal humans. However, mouse serum uridine levels after PALA treatment consistently fell to about half of the normal range which may help to explain, in part, why PALA is curative towards Lewis lung carcinoma cells in BDF₁ mice but is ineffective in humans.

A new study was initiated to examine the relative use of de novo pyrimidine biosynthesis versus salvage of pyrimidine nucleotides by cells in tissue culture. The incorporation of ¹⁴C-bicarbonate into uracil nucleosides of L1210 cells was determined under conditions in which the uridine concentration in the medium was maintained constant. When uridine levels in the medium were maintained at 0.3-2.0 μ M, incorporation of ¹⁴C-bicarbonate was 14% of controls, and was reduced to less than 3% of controls at medium uridine levels of 10-12.5 μ M. These results indicate that at normal serum levels L1210 cells depend mainly on extracellular pyrimidines rather than newly synthesized pyrimidines to maintain their pyrimidine pools. If this is also the case for tumor cells in vivo, then the lack of therapeutic response by humans towards inhibitors of de novo pyrimidine biosynthesis may be explained by a reduced dependency of tumor cells on the de novo pyrimidine biosynthetic pathway.

To facilitate biochemical investigation of pyrimidine regulatory activity of the liver, studies were initiated to trace [¹⁴C]-bicarbonate incorporation into the uridine nucleotide pool of isolated rat hepatocytes. In addition, a gas chromatographic-mass spectrometric method was developed for measurement of the incorporation of a stable isotopically labelled precursor of the de novo pyrimidine biosynthetic pathway into the acid soluble uracil-nucleotide pool of L1210 cells. The method developed for measurement of [¹³C]-bicarbonate incorporation into the nucleotide pool of L1210 cells in vitro was compared with the standard technique of incorporation of radioactively labelled bicarbonate into the same cells. The incorporation of the stable and radioactive isotopes into the acid soluble uridine nucleotide pool of L1210 cells was the same, indicating that the GC-MS method was analogous to the more common tracer technique which uses radiolabelled precursors. The results from this study gave evidence of compartmentalization of uridine nucleotides in L1210 cells. The rate of bicarbonate incorporation was linear for only the initial 20 minutes and

a maximum of only 50-60% of the nucleotide pool could be labelled. This indicated that de novo synthesis supplies only half of the total nucleotides and these newly synthesized nucleotides may be utilized by specific RNA species. The remaining nucleotides in the pool must be supplied by salvage and reutilization of breakdown products. The stable isotope incorporation technique is currently being used to measure the flux through the de novo pyrimidine biosynthetic pathway in vivo.

Studies on the therapeutic effects of several combined immuno therapeutic modalities were performed during the past year. A series of maleic anhydride ether (MVE) polyanions, synthesized with molecular weights ranging from 12500 to 52600, were found capable of enhancing macrophage tumoricidal activity against MBL-2 leukemia cells. These agents also augmented natural killer (NK) cell activity against the YAC lymphoma and M109 adenocarcinoma cell lines. This response appeared to be dependent upon MVE's ability to activate macrophage tumoricidal activity. When these agents were combined with cyclophosphamide an enhanced antitumor (curative) response was achieved. This enhancement may be attributable to the effective tumor cytoreductive response to cyclophosphamide followed by an augmented immunological response by MVE through the action of activated macrophage tumoricidal effect on residual tumor cells. The MVEs were examined further for their capacity to act as adjuvants to tumor cell vaccines. Augmentation of the immune response was achieved when the MVEs were combined with irradiation killed vaccines of L1210, LSTRA (leukemias), and the B16 melanoma. Protection against live tumor cell challenge was found to be tumor cell specific. No cross-protection was achieved. Macrophage involvement in antigen processing of the tumor cell vaccine was demonstrated when carrageenan, a macrophage inactivator, was shown to abort the protective effect obtained with vaccination and MVE treatment. B cell participation was considered obligatory since vaccinated mice, surviving live tumor cell challenge, were also shown to reject a second live tumor cell challenge several weeks later.

The M109 alveolar carcinoma, a tumor which is refractory to several chemotherapeutic agents, was also employed to assess the effect of combined chemo-immunotherapy. A marked reduction of lung tumor lesions was achieved, resulting in a longer remission period and a significant number of long term survivors, with combined BCNU chemotherapy and MVE immunotherapy. Ancillary studies indicated that this treatment was successful because the primary chemotherapy reduced the tumor burden sufficiently so that tumoricidal macrophages, activated by the secondary MVE treatment, further reduced residual tumor cells in the lung.

Poly-ICLC was tested in mice for its immunoregulatory activity. It was found to enhance T cell responsiveness but not B cells, and augmented the delayed type hypersensitivity response significantly. Macrophage tumoricidal activity was markedly enhanced both in vitro and in vivo after exposure to Poly-ICLC. NK cell cytotoxicity was significantly augmented in vivo. Both macrophage and NK cell activity was maintained for over 3 days after only one treatment. The extended period of tumor cell cytotoxicity exhibited by macrophages and NK cells may correlate with Poly-ICLC induction of early and high levels of interferon which are maintained in the serum for a longer period of time.

In a different area, studies were continued on the ability of some chemicals to induce malignant cells to differentiate and on the mechanism by which this process occurs. The chemicals evaluated included hexamethylene bisacetamide (HMBA), DMSO, dexamethasone, theophylline and butyric acid (BA). Two hepatoma

cell lines McA-RH 7777 (7777) and McA-RH 8994 (8994) were treated with HMBA to assess its effects on the production of alpha fetoprotein (AFP), albumin and transferrin. Radioimmunoassays were used to determine the levels of both secreted and intracellular concentrations of AFP, albumin and transferrin. The cell lines 7777 normally produces large amounts of AFP but the production of albumin is very low in this cell line, and it therefore resembles the fetal liver with respect to the secretion of these two proteins. In contrast 8994 produces large amounts of albumin and very small quantities of AFP thus resembling hepatic functions characteristic of a more differentiated state. After a period of 28 to 36 hours of HMBA treatment a 3 fold increase in AFP secretion by 7777 and a dose related increase in AFP, albumin and transferrin secretion by 8994 were observed. However, in 7777 HMBA treatment decreased the secretion of albumin and transferrin to 40% and 75% respectively of control values. The intracellular concentration of AFP in 7777 and all three proteins in 8994 was increased by treatment with HMBA indicating that this agent is able to stimulate the synthesis of these proteins. The intracellular concentration of transferrin produced by 7777 was decreased by treatment with HMBA indicating an inhibition of transferrin synthesis. However, while secretion of albumin was decreased by HMBA treatment of 7777 intracellular albumin increased in this cell line indicating a limited secretory capacity for this protein and/or a stimulation of albumin synthesis which is masked by a limited secretory process. HMBA stimulates the production of the oncofetal protein AFP, a result which appears to be independent of the stage of differentiation of the cell. However, its effect on albumin and transferrin are opposite in the two cell lines, suggesting that the production of these two proteins are controlled by factors or conditions which are dependent upon the stage of differentiation of the cell.

Also under study are several unique undifferentiated lymphoma cell lines which are EBV negative and contain the 8q, 14q translocation. The lines have been analyzed for the presence of surface and cytoplasmic Ig in addition to surface receptors for EBV, complement (C), and the Fc portion of IgG; in addition, 2 dimensional gel electrophoresis was employed to compare patterns of cellular proteins among the cell lines. The lines differ considerably in expression of cell surface markers and their ability to be induced for the same with pharmacologic agents. Two lines derived from patient JD are of particular interest; one line derived from the original tumor JD-A and another (JD-PB) was obtained at relapse. JD-PB possesses increased numbers of C receptors compared to JD-A (25% vs 0-10%) and is much more readily infected with EBV suggesting increased numbers of EBV receptors. In addition 2D gel electrophoresis of total cellular proteins demonstrates several differences between the lines. High level of C receptors (50-90% of cells) were readily induced in the JD-PB line by HMBA, DMSO, ascorbate, dexamethasone, theophylline, and BA. Similarly the JD-A line was induced, but to much lesser extent, with HMBA and BA. Theophylline has no effect upon JD-A C receptor induction. These data suggest that the JD-A and JD-PB lines are clones that differ both with respect to stages of differentiation and the capacity to be induced to differentiate by pharmacological agents.

The effects of inducers of cellular differentiation, such as DMSO and BA on membrane microviscosity has been determined by ESR spectroscopy. Friend erythroleukemia cells that had been differentiated by both DMSO and BA showed marked decreases in membrane microviscosity. Preliminary data indicate that microviscosity changes are not a steady transition from control levels to differentiated levels; instead, a decrease in microviscosity appears to occur during the first 24 hours of exposure to the inducing agent after which progressive increases.

in microviscosity are seen.

Changes in gene expression during differentiation of other virally and chemically transformed cells are also being analyzed. Two clones (C19TK and 5-19) of Friend erythroleukemia cells were used to study the terminal differentiation of these cells to hemoglobin producing cells. Both lines can be induced to differentiate to hemoglobin producing cells following treatment with a wide variety of chemicals, including DMSO, HMBA, and BA. In addition, erythro-differentiation induced by these chemicals can be blocked by hydrocortisone (HC) and dexamethasone (Dex); the β adrenergic blocker, dl-propranolol is also an excellent inhibitor of both DMSO and HMBA induced erythrodifferentiation in 5-18 cells but not in C19TK cells. Qualitative and quantitative changes in protein patterns from total cellular extracts (both membrane and cytosolic proteins) from untreated cells and cells treated with DMSO, HMBA, DMSO/HC, DMSO/Prop, HMBA/HC, and HMBA/Prop are being assessed using 2-dimensional electrophoresis.

The toxicity, as well as the therapeutic properties, of agents used to treat cancer has been evaluated during the past year. Utilizing a rat myocyte system developed in this laboratory, and described in last years Annual Report, the cardiotoxic potential of several anthracyclines, two anthracenediones, and m-AMSA has been tested. m-AMSA is toxic to the beating cells in a manner very similar to that of adriamycin, but the anthracenediones were not toxic at the limits of their solubilities (greater than 10^{-2} M). Two anthracyclines, AD-32 and aclacinomycin A, were not toxic at the maximal concentrations tested. They were also the only two anthracyclines evaluated that did not appear to bind to nuclei of the heart cells as evaluated in viable cells with the fluorescence microscope.

Studies on the renal and intestinal toxicity of cisplatin were continued. Cisplatin nephropathy and renal accumulation of platinum were analyzed in rats after both acute and chronic treatment. A single i.p. dose of cisplatin (6 mg/kg) induced marked focal necrosis in the proximal and distal tubules with a maximum lesion on day 7. The tubular damage was localized mainly in the corticomedullary region, where the concentration of platinum was the highest within the kidney. Repeated treatment with cisplatin (1 mg/kg, i.p., twice weekly) for 11 weeks resulted in massive tubular dilation in the corticomedullary region, interstitial fibrosis and thickening of tubular basement membranes. Some glomeruli appeared fibrotic, indicating that chronic treatment with cisplatin could cause irreversible renal damage. The intestinal cytotoxicity of cisplatin was qualitatively and quantitatively characterized in rats following a single ip dose (5 mg/kg). Cellular necrosis and inhibition of mitosis in the intestinal epithelium were maximal on days 1-2 and were most severe in the ileum, but mucosal lesions were recovered by 5-7 days. Crypt and villus cell populations were reduced most in the ileum (60-70%), followed by the jejunum (45-60%), and the duodenum (35-40%). Stomach and colon had few mucosal lesions. GI tissues assayed for cisplatin indicated no preferential localization of cisplatin in any segment of small intestine. Histologic evidence suggested that proliferating epithelial cells in the crypt are the major targets for cisplatin cytotoxicity. Currently experiments are underway to determine if orally administered platinum-binding compounds (i.e., $\text{Na}_2\text{Ca-EDTA}$) can ameliorate the intestinal toxicity of cisplatin.

The oocyte and follicle toxicity of several antineoplastic and immunosuppressive agents (cyclophosphamide, azathioprine and 6-mercaptopurine) was studied in a

murine ovarian toxicity system. Seven days after a single treatment with cyclophosphamide (100 mg/kg, ip), 63% of the primordial follicles and 0% of the growing or large follicles and their oocytes were destroyed in four week old C57BL/6N mice. Treatment with azathioprine (100 mg/kg/day, ip x 9 days) or 6-mercaptopurine (100 mg/kg/day, ip x 9 days) suppressed weight gain and increased mortality but had no effect on the number of oocytes or follicles in these mice. These data are consistent with previous observations on the effects of cyclophosphamide, azathioprine and 6-mercaptopurine on the human ovary. This suggests that the murine ovarian toxicity assay system may be useful in evaluating xenobiotics, including drugs, for human ovarian toxicity.

In a study initiated this year, the possibility of preventing drug-induced infertility in males by suppressing spermatogenesis prior to, and during, administration of cyclophosphamide (CTX) or procarbazine (MIH) is being evaluated. Both of these agents are known to induce infertility in human and rodent males, presumably by their non-selective action against rapidly proliferating cells, including spermatogonial stem cells. The rationale of this study is that suppression of spermatogenesis by sex steroids will induce a nonproliferative phase in the primary spermatogonia and that during this phase they will be less vulnerable to the toxic effects of CTX and MIH. Testosterone-containing capsules were implanted 3 week prior to initiation of treatment with CTX or MIH; control groups included mice implanted with capsules and receiving saline injections, and mice without capsules injected with CTX or MIH. Drug doses were chosen to correspond to those used in clinical situations and drug treatment was continued for 10 weeks, which corresponds to 2 spermatogenic cycles in the mouse. At the end of the treatment period, the testosterone capsules were removed, and a subset of mice sacrificed for quantification of serum FSH, LH and testosterone levels and for testicular histology. Additional subsets of animals will be sacrificed for the same purpose at 4 further 8-week intervals. The remainder of the mice will be used in serial mating studies beginning 8 weeks after the end of drug treatment. These mating studies will be carried out to assess fertility in all groups treatment on the survival, frequency of malformations and tumor incidence in their offspring. The segment of the study involving testicular suppression and treatment with CTX and MIH has been completed, and serial mating studies will begin shortly.

This laboratory has had a long-term commitment to studies of the mutagenic and carcinogenic properties of antitumor agents and other model compounds. The mutagenicity of N-hydroxy-2-acetylaminofluorene and N-hydroxyphenacetin and their respective deacetylated metabolites, N-hydroxy-2-aminofluorene and 2-nitrosofluorene, and N-hydroxyphenetidine and p-nitrosophenetole was determined in nitroreductase deficient Salmonella tester strains TA 98FR and TA 100FR. The mutagenicity of N-hydroxy-2-acetylaminofluorene mediated by either rat liver microsomes or rat liver 105,000g supernatant fractions was no different in either TA 98 (nitroreductase present) or TA 98FR (nitroreductase deficient) strains. Similarly the mutagenicity of N-hydroxyphenacetin mediated by hamster microsomes was not affected by either the presence or absence of nitroreductase activity in TA 100. N-hydroxy-2-aminofluorene and 2-nitrosofluorene were equipotent direct acting mutagens in both TA 98 and TA 98FR, as were both N-hydroxyphenetidine and p-nitrosophenetole in TA 100 and TA 100FR. Ascorbate (5 mM) and NADPH (1 mM) had no significant effect on the mutagenicity of either N-hydroxy-2-acetylaminofluorene, or 2-nitrosofluorene in TA 98 or TA 98FR whereas ascorbate and NADPH markedly inhibited the mutagenicity of both N-hydroxyphenetidine and p-nitrosophenetole in both TA 100 and TA 100FR. Ascorbate appears to be

inhibiting the mutagenicity of N-hydroxyphenetidine and p-nitrosophenetole as a result of the non enzymatic chemical reduction of these compounds to non mutagenic derivatives.

Coincubation of isolated intact rat hepatocytes and *Salmonella typhimurium* tester strains (*Salmonella*/hepatocyte system) permits determination of both bacterial mutagenicity and DNA damage in the hepatocyte as measured by alkaline elution following treatment with known or suspected chemical carcinogens. The use of the esterase inhibitor paraoxon in the classical *Salmonella* system indicates that deacetylation of N-OH-AAF is the primary mutagenic activation pathway for this compound. The effect of paraoxon pretreatment on both bacterial mutagenicity and host cell DNA damage of N-OH-AAF was determined in the *Salmonella*/hepatocyte system. Pretreatment with paraoxon over a concentration range from 10^{-4} to 10^{-6} M caused dose related inhibition of the bacterial mutagenicity of N-OH-AAF. However, the effect of paraoxon on N-OH-AAF induced DNA damage was inhibitory at concentrations lower than 10^{-6} M but at high concentrations of paraoxon the DNA damage was accentuated. These data indicate that paraoxon at low concentrations inhibits both bacterial mutagenicity and host cell DNA damage by N-OH-AAF in the *Salmonella*/hepatocyte system. The host cell DNA damage of N-OH-AAF is increased at higher concentrations of paraoxon whereas the bacterial mutagenicity of N-OH-AAF is still inhibited.

The availability of a large breeding and experimental colony of nonhuman primates (500 animals representing 4 species) continues to provide this laboratory and other cooperating units with a unique resource for comparative pharmacologic, toxicologic, biochemical and carcinogenesis studies. The carcinogenic effects of 27 substances, including antineoplastic and immunosuppressive agents, model rodent carcinogens, pesticides, artificial sweeteners and contaminants of human foodstuffs are currently being evaluated. Eight of the 27 substances evaluated have induced malignant neoplasms in nonhuman primates, producing a tumor incidence ranging from 9.1-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B₁, methylazoxymethanol acetate, procarbazine, methylnitrosourea, and urethane. In addition, single cases of malignant tumors have been diagnosed in animals treated with adriamycin, N-methyl-N'-nitro-N-nitrosoguanidine and N,N'-dimethyl-p-phenylazoaniline (butter yellow).

Since the inception of this study 20 years ago, 7 spontaneous tumors have been diagnosed in 219 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of 3.2%. Of the 27 substances entered on test during this period, 16 [3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, N,N'-dimethyl-p-(m-tolylazo)-aniline, cyclamate, saccharin, dichlorodiphenyltrichloroethane, low density polyethylene plastic, cigarette smoke condensate, arsenic, sterigmatocystin, Imuran, L-phenylalanine mustard and cyclophosphamide] have not as yet induced tumors. However, several of these compounds have been under evaluation for only a short time period.

Cyclamate has been under test for the past 10-1/2 years. Two groups of monkeys have received this compound orally, 5 days every week, at 100 and 500 mg/kg, respectively. The 100 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 2.3 gm/day/70 kg man, and is equivalent to drinking about 6 diet drinks per day. The 500 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 11.6 gm/day/70

kg man, and is equivalent to drinking about 30 diet drinks per day. Two of 12 monkeys at the low dose, and 2 of 11 monkeys at the high dose have been necropsied, but no evidence of a malignant neoplasm was found.

Two groups of 10 monkeys each have been receiving oral doses of saccharin (25 mg/kg), 5 days every week. This dose corresponds, on an equivalent surface area basis, to a daily intake of 5 cans of diet soda by a 70 kg man. One group of monkeys has been receiving saccharin for an average of 122 months (range 120-124 months), and the second group of 10 monkeys began saccharin treatment approximately 3 years ago. Since the inception of the study, none of the monkeys have died, and there is no evidence of toxicity in any of the treated animals.

Similarly, long-term administration of DDT has not resulted in the development of tumors in nonhuman primates. A total of 15 animals have received DDT by the oral route (20 mg/kg) daily, 5 days every week in a study that has been underway for the past 134 months. Administration of DDT is discontinued after a dosing interval of 130 months is completed. Although 5 of the monkeys have died thus far, none were found to have developed tumors. The apparent cause of death in these animals was DDT-induced CNS toxicity, as all experienced severe tremors and convulsions immediately prior to death. The 10 surviving monkeys appear to be in good health.

In contrast to these chemicals, DENA is highly predictable as a hepatocarcinogen in Old World monkeys, and accordingly a relatively large amount of information on its carcinogenic effects has been accumulated in these animals. DENA has induced tumors in 33 out of 45 Old World monkeys receiving oral treatment with 40 mg/kg 5 times a week. These tumors, all of which were hepatocellular carcinomas, developed in rhesus, cynomolgus and African green monkeys which had received the initial dose of DENA either at birth, at 1-8 months postpartum, or as adults. There is some indication that monkeys receiving the initial dose of DENA at birth required less total DENA for tumor development than those monkeys in which initial treatment was delayed until 1-8 months postpartum or until adulthood. However, the number of animals in each group is not sufficiently large to test the statistical significance of this difference. An apparent species difference exists with respect to both latent period and cumulative dose for tumor induction. The latent period averaged 26, 49 and 105 months for cynomolgus, rhesus and African green monkeys, respectively. The average total DENA dose ingested by cynomolgus monkeys developing tumor was 18.0 gm; for rhesus monkeys this value was 25.4 gm, and for African greens it was 55.1 gm. This species difference was not noted, however, when DENA was given by the ip route. A total of 106 cynomolgus, rhesus, African green and rhesus x cynomolgus hybrid monkeys given bimonthly doses (40 mg/kg) of DENA developed hepatocellular carcinomas. The latent period averaged for the 4 species was 14 months (range 15-17 months) and the average total dose of DENA necessary for tumor development was 1.7 grams (range 1.32-1.95 gm). The latent period and the total dose required for tumor induction by the ip route appeared to be independent of the age at which dosing was initiated.

DENA is also carcinogenic in the more primitive primate Galago crassicaudatus. All 10 treated animals have developed tumors after bimonthly ip injections of DENA at doses of 10-30 mg/kg. In contrast to the DENA-induced primary hepatocellular carcinomas in Old World monkeys, all 10 of the bushbabies developed mucoepidermoid carcinomas of the nasal cavity. In 2 of these 10 animals, car-

cinomas of the liver were also present, and in both cases metastases to the lungs or to intestinal lymph nodes was noted. The average total dose of DENA given the bushbabies was 0.747 gm, and ranged from 0.295-1.485 gm. The latent period averaged 20 months (range 12-27 months).

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving 1-nitrosopiperidine (PIP) by the oral route, and in 5 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given orally (1742.5 gm) was higher than for oral DENA (18.0-55.1 gm); similarly, the average cumulative dose of PIP given by the ip route (39.4 gm) exceeded that required for tumor induction by ip DENA (1.7 gm) or ip DPNA (7.0 gm).

DENA is currently being used as a model carcinogen to investigate two questions of importance to chemical carcinogenesis. The first question pertains to whether there is a specific total dose of carcinogen which, within the lifespan of the test animal, will induce a tumor. In order to evaluate this question, groups of monkeys are being given bimonthly ip injections of DENA at doses of 0.1, 1, 5, 10, 20 and 40 mg/kg, and are observed for the appearance of tumor. In the 4 groups of monkeys (40, 20, 10 and 5 mg/kg doses) in which tumors have developed, it was found that the latent period increases as the mg/kg dose decreases. Thus with the 40 mg/kg dose the latent period is 17 months, whereas at 5 mg/kg it is 65 months. The study is as yet incomplete and in some groups the proportion of tumor-bearing animals is small, so that it is not yet possible to report a precise value for the carcinogenic dose of DENA. However, it appears that this value will lie between 1.4 and 3.0 gm.

The second question pertains to whether chemically-induced cancer can be prevented or reversed by other chemicals, either administered simultaneously with the carcinogen, or before carcinogen exposure. It is now recognized that the reactive forms of most chemical carcinogens are strong electrophiles and are capable of attacking tissue nucleophiles. Thus, one of the possible approaches to the prevention or reversal of chemical carcinogenesis is to supply an excess of non-critical nucleophiles, thereby "scavenging" the reactive electrophilic species of carcinogens once formed. This approach is being employed in 2 groups of monkeys receiving bimonthly ip doses of DENA at 10 mg/kg or 5 mg/kg. In both groups, the DENA dose is given 10 minutes after an ip injection of a "protective cocktail". For the monkeys receiving DENA at 10 mg/kg, the "protective cocktail" consists of L-cysteine (20 mg/kg), cysteamine (20 mg/kg) and reduced glutathione (150 mg/kg). The "protective cocktail" given the monkeys receiving DENA at 5 mg/kg is composed of N-acetylcysteine (50 mg/kg). It is possible that these sulfhydryl reagents will prevent the covalent binding of the activated form of DENA to critical macromolecules of liver tissue, an event which is thought to be a prerequisite for hepatocarcinogenesis by a variety of chemicals including DENA. However, during the course of studies with both "protective cocktails", it became apparent that they were producing a significant degree of peritoneal irritation; necropsy of several of the animals revealed intestinal perforation or obstruction attributable to multiple peritoneal abscesses and adhesions. For this reason, dosing with DENA and the "protective cocktails" was discontinued. In the interim, 8 of 10 monkeys receiving DENA (10 mg/kg) and the "protective

cocktail" have developed tumor. When dosing with DENA (5 mg/kg) is resumed, N-acetylcysteine will be given by the oral route rather than ip.

Monkeys bearing DENA-induced hepatocellular carcinomas have also been used in the development and the experimental evaluation of a new contrast medium for computed tomographic (CT) examination of liver and spleen. This lipoid-based contrast material (EOE 13) contains 53% ethiodized oil in emulsion form. In normal monkeys, an intravenous dose of 0.2 ml/kg selectively opacified the liver and spleen, resulting in an average increase of 23 EMI units (500 scale) in the attenuation of the liver and a higher increase in the attenuation of the spleen. When injected into rhesus monkeys with hepatocellular carcinoma, there was a significant improvement in the visualization of the tumor, and small lesions, undetectable on the preliminary CT scan, became visible. Toxicity studies have been completed in animals and the contrast material is in clinical trials at the Clinical Center. Ten patients with disseminated cancer were given intravenous injections of 0.2 ml/kg (40 mg I/kg) of EOE 13. CT scans of the liver and spleen were taken prior to and 30 minutes after contrast infusion. Visualization of the liver was significantly improved in 5, moderately improved in 3, and not appreciably improved in 2. The spleen showed an obvious increase in density in all cases. No significant toxicity was encountered: untoward side effects consisted of fever, headaches, foul metallic taste, and weakness for a short period. Four patients had no side effects, and 2 experienced only abnormal taste sensation. Additional experimental and clinical work is needed to further document the advantages and safety of this contrast material.

In addition to the Laboratory research effort, the senior investigators are involved in the teaching and training of visiting fellows, medical students, graduate students, staff fellows, and clinical associates. In addition, investigators from outside NIH spend short periods of time in our laboratory in order to learn specific techniques. Some of the senior investigators are also responsible for the administration of contracts with outside research institutions. The contracts are concerned with evaluation of various aspects of the adverse effects of antitumor agents, and clinical pharmacokinetic studies of new antitumor agents. Agents under evaluation in the latter contract are of high priority for clinical development in the Developmental Therapeutics Program and the Division of Cancer Treatment.

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COOPERATING UNITS (if any) Digestive Diseases Branch, NIAMDD; Biometry Branch, NIMH; Lab. of Exp. Pathol., NCI; Lab. of Cerebral Metabolism, NIMH, Surgical Neurology Branch, NINCDS, Dept. of Neurology, Cornell University; Dept. of Neurology, Northwestern University; Dept. of Neurosurgery, National Naval Medical Center																																																												
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SUMMARY OF WORK (200 words or less - underline keywords) New techniques developed during this year include ways to obtain both <u>quantitative autoradiographic (QAR)</u> and histological images from the same tissue section (<u>co-imaging</u>) and to perform simple chemical extractions of radioactive materials on QAR sections to determine the extent of <u>tissue binding</u> . <u>Blood-tissue distribution of misonidazole</u> , a radiosensitizer, apparently is limited mainly by <u>blood flow within tumors</u> . The <u>inhalation of 20% CO₂</u> appears to modestly open the <u>blood-brain barrier (BBB)</u> to water-soluble, relatively impermeable compounds. In studies with the <u>ASV primary brain tumor model</u> , <u>blood flow (F)</u> was sharply diminished only in the large tumors, whereas the <u>blood-tissue transfer rate constant of AIB (K_t)</u> was clearly increased in both large and small tumors. <u>Glucose utilization (R)</u> by the ASV tumor was relatively normal in the small tumors but elevated nearly two fold in one large tumor. The <u>uptake and binding of tracer thymidine</u> in brain and ASV-induced brain tumors was limited; significant binding occurred in some, but not all, large tumors. All of our studies indicated marked differences between and within these tumors for F, K _t , and R as well as misonidazole and thymidine distribution.																																																												

Project Description:Methods Employed:

Most of our experimental work is done upon in vivo systems and involves the administration of radioactively labelled materials into the vascular compartment, repeated sampling of the blood, killing the animals at various times, and obtaining tissue and fluid specimens for radiotracer analysis. Since the organ system of major interest to us is the central nervous system (CNS), we seek experimental procedures for investigating physiological, pharmacological, and biochemical processes which cause minimal perturbations of the state of the animal and the CNS function or system we are examining. To accomplish this goal, ways of performing these types of experiments on unanesthetized animals have been adopted.

Rats are generally used for the vascular infusion (blood-to-tissue) studies. The procedure consists of inducing anesthesia with halothane, rapid cannulation of the femoral arteries and veins through a small incision, covering the wound with xylocaine jelly, wrapping the animal's lower quarters in plaster, securing the animal by its plaster cast to a lead brick, and removing the halothane gas, thus allowing the return of consciousness. The animals quickly recover, seem alert and comfortable, and rapidly reach normal awake levels of blood gases and pressure. Labelled materials and drugs can be injected through the venous catheters and blood samples and pressure readings can be easily obtained via the arterial catheters.

For some of the intravascular infusion studies, a mixture of five different radioisotopes was intravenously administered and all tissue and fluid samples were assayed for each isotope by a combination of gamma and beta counting. This permitted the simultaneous measurement of five different parameters--for example, blood flow, vascular space, and blood-tissue transport of three solutes--in a single animal.

For the remainder of the intravenous administration experiments, mainly those involving pathophysiological studies, the tissue concentrations of various ^{14}C -labelled substances were determined by quantitative autoradiography (QAR). With this technique, "microdissection" of organs and physiological processes can be carried out with a resolution of about 100 microns. Attempts to expand this methodology to other radioactive isotopes have been successful. Several Auger electron emitting isotopes have been found to produce reasonably sharp radioautographs and useable standards for quantitation. Subsequently, two protein molecules have been labeled with these isotopes, namely ^{111}In -transferrin and ^{125}I -human serum albumin, and used for the various studies.

Currently we are using quantitative autoradiography to measure a number of different processes in the intact animal. Cerebral or other tissue blood flow is determined from the distribution of ^{14}C -, ^{125}I -, or ^{131}I -iodoantipyrine, local glucose utilization by the uptake of ^{14}C -2-deoxyglucose, and protein turnover by the accumulation of ^{14}C -leucine. Measurements of capillary and cellular permeability are made with a number of physiological markers such as ^{14}C - α -aminoisobutyric acid (AIB) and ^{14}C -sucrose. We are currently studying the distribution of ^{14}C -methotrexate, ^{14}C -thymidine, and ^{14}C -misonidazole in normal and

tumor-bearing animals by quantitative autoradiography.

In the past year two different ways of doing double-label quantitative autoradiography have been developed in our section. For both approaches, one isotope is ^{14}C and the other is an Auger electron emitting nuclide of relatively short half-life, for example, ^{125}I . For the first technique, a thin mylar sheet is placed between the tissue section and the X-ray film. The mylar film absorbs all of ^{14}C -radiation but only a small portion of the more energetic Auger electrons, which subsequently interact or expose the X-ray film. After the period of Auger electron exposure is over and sufficient time has elapsed for the Auger electron emitter to decay away (15 half-lives), a new X-ray film without the mylar absorber is placed over the tissue sections and exposed for the detection of ^{14}C , thus producing a second autoradiographic image.

A second technique for performing double label quantitative autoradiography has been tried with some success. This methodology was presented in last years annual report and was not used this year for any studies; therefore it will not be described further.

Since only ^{14}C -methylmethacrylate standards are commercially available for QAR, a method had to be devised for making standards for the auger-emitting nuclides used in our autoradiographic studies. A new technique, which is better than the one reported last year, has been devised. Homogenates of brain or other organs are prepared in a tissue grinder. A stock solution containing the radioactive material in sufficiently high concentration to extend beyond the range of radioactivity anticipated in the actual experiments is made up and mixed with part of the tissue homogenate. A portion of this mixture is drawn into a 1 ml disposable syringe from which the tip has been cut, and the syringe is placed in liquid nitrogen to freeze. The frozen cylinder or stick of radioactive material is expelled from the syringe by the plunger, is mounted on a cryostat planchet, and becomes the highest activity standard for the series. Another aliquot of the starting tissue-radioactive solution mixture is diluted further with unlabelled tissue homogenate. This is then drawn into a second cut-off syringe, frozen, and mounted, thereby making the next standard in the series. This procedure is repeated until 8-10 tissue sticks of diminishing radioactivity have been formed. This series constitutes a standard series and is sliced in the cryomicrotome at the same time that the tissues from the experimental animals are sectioned.

Two new variations of the QAR methodology have been devised by our group. One of these variations enables us to do simple solvent extractions on the frozen and dried tissue section and determine the amounts of soluble and bound carbon-14 or other radioactive nuclide present in the tissue; this has been employed in our studies of the distribution of ^{14}C -leucine and ^{14}C -thymidine. The other variation allows us to histologically prepare for microscopical examination the very same tissue section which was used for QAR (in the past the histological data was gained from the fixing and staining of the tissue section adjacent to the one taken for QAR); this present procedure makes for more precise correlations between the morphology and physiology of the tissue section.

During the past year, pathophysiological studies have been made on five different brain tumor models. The first is the Walker 256 metastatic carcinoma model of Ushio, Chernik, and Shapiro. For this model tumor cells are injected into one carotid artery of a rat, and they subsequently lodge throughout head and neck tissues. After about two weeks, the animal is treated with intravenous cyclophosphamide which eradicates all tumor cells except those in the brain. Weight loss and neurological signs which indicate the presence of CNS tumors appear after an additional 7-14 days.

The second brain tumor model under examination is an ethylnitrosourea induced primary brain tumor of rats. This model was developed by Dr. Jerry M. Rice, Experimental Pathology Laboratory, DCCP, NCI, and previously reported by him.

The third and fourth brain tumor models are provided to us by Dr. Dennis Groothuis, Evanston Hospital, Northwestern University. The first of these models is generated by injecting an avian sarcoma virus suspension into the cerebral cortex of young rats; this procedure usually produces one or more gliomas per rat. The second of these models uses RC-2 astrocytoma cells grown in tissue culture and inoculated into the brains of young rats where they form one or more tumors (these cells can also be injected subcutaneously, thus producing large flank tumors).

Finally, a primary brain tumor model which is being jointly developed by our group and the Surgical Neurology Branch, NINCDS, has been used for some studies. This tumor is produced by implanting a suspension of RT-9 tumor cells, originally derived from a nitrosourea induced astrocytoma, in the brains of young adult rats. After several weeks tumor masses grow within and around the brain. In addition, RT-9 cells have been subcutaneously implanted in rats to generate flank tumors.

There are reports that the BBB transfer of solutes is increased under conditions of hepatic failure. We are employing a rabbit model of acute hepatic failure to autoradiographically examine the possible changes in cerebral blood flow and AIB transfer as a way of learning more about the biochemical nature of the BBB. This model was developed in the Digestive Diseases Branch, NIAMDD, and is produced by the intravenous administration of galactosamine hydrochloride.

Two techniques which are believed to "open" the blood-brain barrier have been investigated. One of these is the hyperosmotic approach of Rapoport in which a 30 second intracarotid infusion of a 1.6 M solution of arabinose is employed in anesthetized rats; the other procedure used a two hour exposure of the animal to 20% CO₂.

Several mathematical approaches, computer systems, and programs are used to analyze our experimental data. For studies of blood-tissue exchange of various solutes and drugs, three methods of data analysis--the simple arterial integral, the graphic arterial integral, and the K_1/k_2 --are used. All three are relatively new and were described in the 1979 annual report in detail. For the studies of glucose utilization and protein turnover, the approaches of Dr. Louis Sokoloff and coworkers, Laboratory of Cerebral Metabolism, NIMH were employed. The standard equations and computations as originally set up by Kety were used for

the blood flow calculations.

Finally, a sizable amount of time has been spent reading the literature and performing trial experiments, with the intention of finding the best way or ways of studying drug transfer across the blood-brain barrier. In addition, theoretical and computer analyses of this problem have been made by us in collaboration with Dr. C. S. Patlak, NIMH.

Major Findings:

Our review of the transfer of drugs across the BBB indicates that one of three different constants is determined in all studies. They are the extraction fraction (E), the blood-to-brain transfer constant (K_1), and the so-called permeability constant (k_1). The latter is most commonly used in drug studies but provides the least information about blood-brain barrier permeability, the parameter its name implies that it measures. Rather, k_1 (also known as the time constant because its units are reciprocal time) is an indicator of the disposition of a material once it passes across the BBB; therefore k_1 depends upon the compartmentation (cellular or extracellular; passive or active uptake) and metabolism (bound and/or free metabolites) as well as the rate of backflux of the parent compounds and its labelled metabolites across the BBB. Clearly, the evaluation of a drug's BBB permeability from k_1 measurements are fraught with uncertainty, a point we have emphasized in a recent review of methodologies.

The quantification of BBB permeability is relatively straightforward and accurate if either E or K_1 is measured; both of these constants are indicators of unidirectional flux from blood-to-brain. The extraction fraction, E , is measured in single pass experiments and is dimensionless; three different single pass methodologies are currently available, namely, the indicator dilution, the brain uptake index, and the external registration techniques. The blood-to-brain transfer constant, K_1 , is determined in long-term, multi-passage experiments and has the same units as clearance and blood flow (for example, $\text{ml g}^{-1} \text{min}^{-1}$); K_1 is measured in intravenous injection and/or infusion experiments.

Many experimentalists consider that K_1 and E are synonymous with permeability (P) or permeability-surface area product (PS) of the BBB. They are, in fact, functions of both PS and blood flow (F); for example, several derivations have shown that $E = 1 - \exp(-PS/F)$. In addition, K_1 and E are related to each other by a simple expression: $K_1 = FE$ (note: F actually equals either plasma or blood flow, depending on the solute's distribution space within the blood perfusing the tissue capillaries).

From these two simple relationships, it can be shown that: when K_1/F (or E) ≤ 0.2 , (i.e., the transfer process is small relative to blood flow), K_1 approximates PS of the BBB for the material of interest; when E (or K_1/F) ≥ 0.95 (i.e., the PS product is three or more times larger than F), the transfer of a material across the BBB is dominated by its rate of delivery to the capillaries, F ; and when E (or K_1/F) lies between 0.2 and 0.95, the unidirectional flux is a function of both PS and F .

The dependency of E and K_i upon PS and F must be appreciated for a clear understanding of drug uptake by the brain under normal and various pathological and experimental conditions. For instance, if a particular CNS lesion increases brain capillary permeability (higher PS products for all solutes) but more markedly diminishes tissue blood flow, a low rate of tissue exchange (a low K_i) would be found, an observation which falsely suggests low capillary permeability; to enhance drug delivery in this situation one should not seek a more permeable drug but rather should find a way to increase blood flow to the target tissue. The preceding considerations have lead us to conclude that not only K_i (or E) but also F must be measured when doing pharmacological and pathophysiological studies of in vivo systems.

Two model-independent ways of calculating K_i of labelled solutes have been established in the past several years. The first of these, single time point analysis, requires only that the test material be effectively trapped in the tissue by a metabolic and/or transport process once it has passed across the capillary endothelium, thereby eliminating backflux of tracer. We have established that such "trapping" does occur for three model substances of markedly different transportability, tracer potassium (^{42}K), alpha-aminoisobutyric acid (AIB), and ^{57}Co -DTPA, over experimental periods of three hours or more in most cases. The K_i 's of these three materials for the normal BBB are: 20×10^{-3} ml/g-min (^{42}K), 3×10^{-3} ml/g-min (AIB) and 0.2×10^{-3} ml/g-min (DTPA) and cover a 100-fold range in blood-brain transfer rates.

The graphic arterial integral approach is the second way of analyzing data and calculating K_i . This analytical method is less model dependent, more general, and more informative than single time point analysis; it enables the user to ascertain the time period over which the uptake process is unidirectional, to measure the size and kinetics of exchange for any fast-loading space (V_e), and to locate the probable site of the BBB. Calculations of K_i and V_e for a large array of materials and conditions have been made utilizing the data from the various experiments performed during the year. These values will be presented with the various studies (vide infra).

Of the five brain tumor models available to us, we have studied the virally-induced glioma (ASV) one most extensively this year. In a double label quantitative autoradiographic study, tissue blood flow was measured with ^{131}I -iodoantipyrine (IAP) and blood-to-tissue transfer with ^{14}C -AIB, and marked regional variations in F and K_i as well as histological appearance were found. In the large tumors (2-10 mm diameter), the K_i 's of AIB were increased 20-150 fold in the center, 6-200 fold at the tumor edge, and several fold over normal brain in the tissue surrounding the tumor; moreover, F was consistently and dramatically diminished in the center of these tumors ($< .06$ ml/g -min), slightly decreased to normal at the edge (.25-1.0 ml/g -min), and definitely diminished in brain tissue around the lesion (.06-.6 ml/g -min). In the small tumors (< 2 mm diameter), K_i was modestly increased throughout (2-20 fold) and was normal in the surrounding neural tissue, whereas F was slightly decreased or normal within the tumor and the surrounding brain. These alterations in K_i and F were correlated more with tumor size than with tumor histology.

Simultaneous measurements of local glucose utilization rate (R) and F were made in a second group of ASV rats using double-label QAR of ^{14}C -2-deoxyglucose (2-DG), and ^{131}I -IAP. The tumors which were found and studied were relatively small (< 3mm in diameter), with one exception, but quite variable in histological appearance. The same relationship between tumor size and F as previously presented for this model system was found. Although differences in R were observed within each tumor, most of them showed a similar range of rates (30-50 μM glucose/100g-min) and patterns of R; however R values greater than 100 μM /100g-min were found in the one large tumor, a polymorphic glioma. The local glucose utilization rate did not seem to correlate with tumor size, histology, cytology, or cellular density in the ASV model.

Local incorporation of ^{14}C -L-leucine (Leu) into a non-extractable tissue fraction, presumably protein, was measured in single-label QAR studies of the ASV tumor system (to the best of our knowledge, this is the first attempt to combine a simple chemical procedure with QAR to obtain information on a biochemical parameter). Leu incorporation varied from very low to very high values within groups of tumor cells with not only similar but also different cytological characteristics. For example, an irregular distribution of Leu-derived- ^{14}C frequently occurred in tumors of uniform histological appearance. The incorporation of Leu appeared to correlate with the nuclear/cytoplasmic ratio within a given tumor and was independent of tumor size and location. Negligible Leu incorporation was always seen in necrotic regions.

The distribution of ^{14}C -thymidine-derived radioactivity (^{14}C -Tdr) in ASV induced brain tumors was determined on untreated and methanol-extracted tissue sections by single-label QAR, and the adequacy of ^{14}C -Tdr as a marker of DNA synthesis within the brain and brain tumors was examined. The non-extracted QAR images showed a general low level of radioactivity throughout the section with some areas of high ^{14}C -activity which corresponded to many but not all of the tumor sites. Methanol extraction of these same tissue sections, a procedure which is purported to remove free Tdr and Tdr metabolites, caused a complete or nearly complete removal of the low level activity throughout the section and some lowering of concentration in all high activity tumor areas. The distribution of the non-extractable radioactivity within the tumors varied from negligible to high values and deviated markedly from the histological findings; a number of tumors which histologically demonstrated pronounced nuclear activity did not display appreciable ^{14}C -concentration. These observed discrepancies between the histological and autoradiographic images suggest that the disposition of non-extracted ^{14}C -Tdr within brain tumors is not solely a function of the rate of DNA synthesis and reflects, to some extent, other factors such as capillary blood flow and permeability.

The local incorporation of ^{14}C -leucine was also studied by QAR in the Walker 256 metastatic brain tumor model. Only small tumors (less than 2mm in diameter) were found in this group of animals. The ^{14}C -activity in most of these tumor sites was higher than in the surrounding brain tissue but the relative tumor: normal brain differences found with ^{14}C -leu were not as great as those reported last year for ^{14}C -2DG (glucose utilization) for the same model system. Methanol extraction of the tissue sections from some of the Leu experiments suggest that the ^{14}C -distribution patterns which were observed reflect protein-bound tracer

and protein synthesis.

Quantitative autoradiographic studies of the RT-9 brain tumor model have been made with AIB, IAP, 2-DG, and Leu, but the densitometric analysis of the autoradiograms has only been partially completed. The following general observations have emerged as of now. Marked local differences in K_1 (AIB experiments) and F (IAP experiments) were found in all tumors observed. The largest values of K_1 and the smallest values of F were always found within the biggest tumors. Great regional differences in the distribution of ^{14}C -2DG- and ^{14}C -leucine-derived radioactivity were noted, but no clear correlation with the size, location, or histology of the several tumors is presently evident.

An investigation of the distribution of ^{14}C -misonidazole (MISO), an hypoxic cell radiosensitizer, in rats with RT-9 gliomas in their flanks was undertaken using QAR and HPLC. A very heterogeneous distribution of MISO-derived radioactivity was observed at the various sampling times. Appreciable amounts of radioactivity were found only around the rim of the tumor 5-10 minutes after bolus intravenous injection. In experiments where constant blood ^{14}C -activities were maintained by the continuous infusion of labelled MISO, the same large discrepancy in concentrations between tumor rim and center was seen. For instance, the ^{14}C -activity in the rim was 4-5 times higher than in the center after 30 minutes. At all tissue sampling times, the disposition of the infused radioactivity was not uniform within the centers of the tumors; there were islands of cell therein with considerably higher amounts of activity than adjacent necrotic areas. Double label QAR experiments with ^{14}C -MISO and ^{131}I -IAP showed that the blood flow (IAP) and MISO distribution patterns were very similar and that delivery of MISO in this tumor system is greatly limited by blood flow. HPLC analysis also indicated that less than 40% of the ^{14}C -activity was associated with the parent drug after 4 hours of MISO infusion. Metabolism, therefore, may also compromise the disposition of MISO within this tumor model.

In a single squirrel monkey with a virally induced brain tumor, measurements of local glucose utilization were made in which both ^{18}F -2DG and ^{14}C -2DG were simultaneously administered and tissue and blood levels of ^{18}F and ^{14}C were separately estimated by positron emission tomography (PET) and QAR, respectively. The local glucose utilization rates measured by the two different techniques were virtually identical in all brain and brain tumor regions and were higher throughout the tumor than in the surrounding brain. This work lends credence to the measurement of local glucose utilization rates in humans by PET scanning (this work was done in collaboration with the Neurological Surgery Branch, NINCDS).

The possibility of "opening" the blood-brain barrier by the inhalation of 20% CO_2 for 2 hours or more was investigated by multiple isotope detection. This treatment had no discernable effect on the blood-brain K_1 's of two substances, the moderately permeable ion, ^{42}K , and the slightly permeable amino acid, AIB; however the transfer across the BBB of DTPA, a virtually impermeable compound, was increased 5-20 fold by the exposure to high blood levels of CO_2 . This peculiar change in BBB permeability suggests that CO_2 inhalation may enhance the exchangeability of very impermeable drugs between blood and brain.

Using the rabbit-galactosamine model, our evaluation of the effects of acute hepatic failure upon brain capillary function indicated that marked changes in BBB permeability to AIB occurred in gray matter 18 hours after drug administration but that no detectable alterations took place in white matter. An earlier study from another laboratory showed that BBB permeability was increased by acute hepatic failure but no gray-white matter differences could be discerned with the technique employed. Our findings suggest that BBB function may be under different control or maintenance systems in gray and white matter.

Blood-to-tissue transport studies of a number of different radioactively labeled solutes have been done in normal rats with the intravascular bolus, multi-isotope technique and analyzed by the graphic arterial integral method. The substances used include amino acids, proteins, and inorganic ions. The K_1 's range from 2×10^{-2} ml/g-min for ^{42}K to 3×10^{-6} ml/g-min for ^{125}I -human serum albumin. The finding that the so-called rapidly exchanging space, V_r , for most of these compounds was bigger than the tissue's blood space was difficult to interpret with a simple single-membrane model of the BBB. As a result we have begun to develop a double-membrane model of the normal BBB, to test the adequacy of this model to explain the fluxes of solutes between blood and brain parenchyma, and to identify the components of V_r .

Significance to Biomedical Research and the Program of the Institute:

A basic understanding of the movements of drugs, physiological materials, and fluids between blood, brain, and CSF is essential for an appreciation of normal CNS functions, of the changes caused in these transport systems by various CNS diseases - especially brain tumors, and of the deviations in these systems which cause neuropathological changes. To perform scientifically reliable transport studies, sound experimental approaches must be used; therefore, as part of our work, various "improved" techniques for measuring and analyzing transport phenomena have been devised. Among these developments are better methods of determining blood-brain transfer constants for materials of low to moderate blood-brain barrier (BBB) permeability, of autoradiographically quantitating several Auger electron emitting nuclides, of doing double-label quantitative autoradiography and of analyzing the data. These various techniques will be used by other research groups who are interested in doing pharmacological, physiological and biochemical studies of the blood-brain-CSF system.

Our studies of glucose utilization, protein synthesis, local blood flow, capillary and cellular transport, and nucleoside distribution in various brain tumor models provide information about the biochemical and physiological changes which occur around and within various kinds of brain tumors and the delivery of anti-tumor agents to CNS neoplasms. These findings will be relevant to human primary and metastatic brain tumors and the design of better treatment protocols and diagnostic procedures for this disease.

The findings provided by our distribution studies of both established and prospective chemotherapeutic agents are needed to determine the best routes and schedules for delivering drugs to the CNS. Without this knowledge effective chemotherapy can be easily thwarted by either inadequate drugs or inappropriate drug administration schedules.

Quantitative autoradiography serves as a model for positron emission tomography; therefore, our efforts to develop ways of measuring various biochemical, physiological, and pharmacological parameters in pathological conditions with quantitative autoradiography may be applied to the study of human disease using PET. In turn, PET studies of human brain tumors and cerebral edema should confirm or deny the applicability of these experimental methods to the study of human pathological conditions and enhance or diminish the predictive value of our animal models.

Since the blood-brain barrier often limits effective CNS drug delivery and chemotherapy, several ways of "beating" the BBB are currently being examined by us. These include not only "opening" the BBB by either the intracarotid injection of hyperosmotic solutions or the elevation of blood and body fluid pCO_2 but also "bypassing" the BBB by administration of the drug into the CSF. In addition, our investigations of disease which appear to open the BBB such as acute hepatic failure may provide information on biochemical ways of increasing the permeability of this membrane system.

Our theoretical analysis and computer modelling of blood-brain-CSF transport systems should be useful for making predictions about the movements of drugs and metabolites in this system and designing effective chemotherapy protocols. Our development of the double-membrane model of the BBB should enhance our understanding of blood-brain transfer in physiology and medicine.

Proposed Course of Project:

The Membrane Transport Section intends to pursue the following research goals for the next year: (1) devise and develop new methods of studying membrane transport and material distribution phenomena, (2) improve and expand the applications and usage of single-label and double-label quantitative autoradiography, (3) gain information about the transport and distribution of various chemotherapeutic agents in normal animals and brain tumor models, (4) study the transport pathophysiology of various primary and metastatic brain tumor models, (5) examine the effects of several different metastatic tissue sites on the biochemistry and physiology of the tumor cells and the tumor mass itself, (6) study the pathophysiological reactions of CNS tissue to other diseases, radiation, and drug treatment, (7) seek information on the normal mechanisms of amino acid, monosaccharide, protein and electrolyte transport between blood, brain and CSF, (8) devise and test a double-membrane model of the blood-brain barrier, and (9) collaborate with other groups in the development of suitable compounds and protocols for PET scanning.

Publications:

1. Blasberg, R. G., Gazendam, J., Patlak, C. S., Shapiro, W. R., and Fenstermacher, J. D.: Changes in Blood-Brain Transfer Parameters Induced by Hyperosmolar Intracarotid Infusion and by Metastatic Tumor Growth. In Eisenberg, H. and Suddith, R. (Eds): Cerebral Microvasculature: Investigations of the Blood-Brain Barrier. New York, Plenum Press, 1980, pp. 307-319.

2. Blasberg, R. G., Gazendam, J., Patlak, C. S., and Fenstermacher, J. D.: Quantitative Autoradiographic Studies of Brain Edema and a Comparison of Multi-isotope Autoradiographic Techniques. In Cervos-Navaros, J. and Ferszt, S. (Eds.): Brain Edema. New York, Raven Press, 1980, pp. 255-270.
3. Blasberg, R., Gazendam, J., Patlak, C., and Fenstermacher, J.: Freeze Lesion of the Cerebrum: Local Capillary Permeability, Blood Flow and Bulk Flow of Edema Fluid. In Schulman, K., Marmarou, A., Miller, J., Becker, D., Hochwald, G., and Brock, M. (Eds.): Intracranial Pressure IV. Heidelberg, Springer-Verlag, 1980, pp. 314-316.
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9. Fenstermacher, J., Blasberg, R., and Patlak, C.: Methods for quantifying the transport of drugs across the brain barrier systems. Pharmacol. Ther. [A] 1981 (in press).
10. Fenstermacher, J. and Gazendam, J.: Intra-arterial infusions of drug and hyperosmotic solutions as ways of enhancing CNS chemotherapy. Cancer Treat. Rep. 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03502-18 LCHP									
PERIOD COVERED October 1, 1980 to September 30, 1981											
TITLE OF PROJECT (80 characters or less) Folic Acid Antagonists in Experimental Tumors and Normal Tissues of Mice											
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: D. S. Zaharko</td> <td style="width: 33%;">Head, Pharmacok. & Pharmacody. Sec.</td> <td style="width: 33%;">LCHP NCI</td> </tr> <tr> <td>Other: S. Creekmore</td> <td>Clinical Associate</td> <td>LCHP NCI</td> </tr> <tr> <td>R. Dedrick</td> <td>Chemical Engineer</td> <td>DEIB DRS</td> </tr> </table>			PI: D. S. Zaharko	Head, Pharmacok. & Pharmacody. Sec.	LCHP NCI	Other: S. Creekmore	Clinical Associate	LCHP NCI	R. Dedrick	Chemical Engineer	DEIB DRS
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COOPERATING UNITS (if any) Biomedical Engineering and Investigation Branch; DRS											
LAB/BRANCH Laboratory of Chemical Pharmacology											
SECTION Pharmacokinetics and Pharmacodynamics Section											
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205											
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SUMMARY OF WORK (200 words or less - underline keywords) A <u>physiological pharmacokinetic model</u> has been developed and constant revision of its <u>parameters</u> is being carried out for its use with <u>MTX</u> and other <u>antitumor agents</u> . Work has begun to attempt to <u>exploit resistance mechanisms</u> in chemo-therapy.											

Project Description

Objectives:

1. To refine a pharmacokinetic model for methotrexate and other antitumor agents and apply it to aid in understanding drug action.
2. To relate pharmacokinetic findings to biochemical and toxic effects when a drug is used alone or in combination with other antitumor agents or endogenous metabolic products.

Methods and Major Findings:

Concentrations of drugs in vivo are often very transient relative to concentrations used in in vitro experiments. Our mathematical model was used to demonstrate that an understanding of whole animal pharmacokinetics permits an assessment of the relative importance of membrane transport in the delivery of drug to the intracellular compartment. The transient nature of the extracellular concentrations can be used to estimate the concentrations and exposure times of intracellular drug. These parameters then can be related to the transient nature of the drug response.

Recent efforts have been directed at determining whether tumor resistance patterns can be used advantageously to increase the therapeutic ratio of certain conventional chemotherapeutic agents. Three basic resistance mechanisms were selected as models for this hypothesis: 1) MTX resistance due to increased levels of

dihydrofolate reductase, 2) MTX resistance due to decreased uptake of drug, 3) adriamycin resistance due to increased efflux of drug. Literature reports indicate tumor models possessing these characteristics are available.

Past studies with thymidine-MTX combinations in our laboratory have indicated that the toxicity of MTX in normal tissues can be reversed with thymidine. We plan to use the substrate homofolate to create a toxic product selectively in those tumors with dihydrofolate reductase above the level blocked by MTX in normal tissues. In principle this should confer a selective advantage to those tissues with the lower dihydrofolate reductase levels - namely the bone marrow and intestinal crypt cells. We are at present establishing baseline toxicities for this combination in normal animals.

In situations where decreased uptake of MTX is the reason for resistance, analogues of MTX that have radioprotective properties may possess a higher therapeutic ratio when used in combination with X-irradiation. It is theoretically possible to synthesize amino alkyl thiol compounds as MTX analogues, and such attempts are currently being made.

Resistance due to increased efflux may be exploited by modification of membrane function with singlet oxygen. Trials in vitro with hematoporphyrin derivatives and photoactivation are underway to determine whether membrane transport can be altered temporarily in this manner. Hematoporphyrin has been reported to fluoresce selectively in certain tumors and has been used with photoactivation to treat skin metastasis of melanomas, carcinomas and sarcomas. It has also been

reported to cause reversible membrane changes after illumination of L1210 cells. We are currently verifying this effect in an L1210 model. We are also doing some preliminary HPLC work on hematoporphyrin in attempts to quantitatively measure this material in biological extracts.

Publications:

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2. Weinstein, J.N., Magin, R.L., Csyk, R.L., and Zaharko, D.S.: Treatment of solid L1210 murine tumors with local hyperthermia and temperature sensitive liposomes containing methotrexate. Cancer Res. 40: 1388-1395, 1980.

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TITLE OF PROJECT (80 characters or less) Potential Embryotoxic, Carcinogenic and other Adverse Effects of Anticancer Agents																						
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SUMMARY OF WORK (200 words or less - underline keywords) The <u>embryotoxic</u>, <u>cytogenetic</u> and <u>carcinogenic</u> effects of new as well as clinically useful antitumor agents are under investigation. Studies on the embryotoxic effects in mice of <u>anthracenedione</u>, <u>dihydroxyanthracenedione</u> and <u>spirogermanium</u> have been completed. The <u>carcinogenic</u> activity of various clinically effective antitumor and/or immunosuppressive agents, including <u>procarbazine</u>, <u>N-methylnitrosourea</u>, <u>melphalan</u>, <u>azathioprine</u>, <u>adriamycin</u>, and <u>cyclophosphamide</u>, is under evaluation in long-term studies in monkeys. A survey of <u>second malignancies</u> developing in treated cancer patients, initiated several years ago, is continuously being updated using data from published reports and from several centers and hospitals. The possibility of <u>preventing drug-induced infertility</u> in males by suppressing spermatogenesis prior to, and during administration of cyclophosphamide or procarbazine is being evaluated in mice.																						

Project Description:Objectives:

This project is designed to obtain data on various aspects of the toxicity of antitumor agents, including their embryotoxic, cytogenetic and carcinogenic effects in rodents, monkeys and in the human. Specifically, the objectives are:

To evaluate in mice the adverse effects on reproduction, including embryotoxic and cytogenetic effects, of various classes of antineoplastic agents. The goal of such studies is to define differences in teratogenic potency among groups of agents with similar mechanisms of antitumor action and antitumor spectra. In addition, the studies are designed to provide information as to the period of embryonic development most sensitive to the effects of the test compounds. The possibility of preventing or reversing antitumor agent-induced embryotoxicity with various chemicals has also been evaluated. In addition, the possibility of protecting the male gonads against antitumor agent-induced sterility by suppressing spermatogenesis during the period of drug treatment is being assessed. Serial mating studies will be carried out in the animals to assess their fertility after treatment, as well as to determine the effects of drug treatment on the survival, frequency of malformations and tumor incidence in their offspring.

To evaluate in monkeys the carcinogenic potential of various clinically useful antitumor and/or immunosuppressive agents. In addition, the effects of such treatment on other functions, such as the immune system and reproduction, is under examination. A significant proportion of monkeys receiving long-term treatment with procarbazine has developed a malignancy, about half of which have been acute leukemia (see below). The surviving monkeys presently receiving procarbazine are considered to be at high risk of developing acute leukemia, and hematologic and cytogenetic studies are currently being carried out in this group of animals. The goal of these studies in the procarbazine-treated monkeys is to determine whether a "preleukemic" condition exists in chemically-induced acute leukemia, and if so, to characterize it.

To monitor the current medical literature and the NCI patient data in order to tabulate and characterize adverse effects of antineoplastic agents in man. Particular emphasis is being given to embryotoxic, mutagenic and carcinogenic effects arising from treatment with clinically effective antitumor agents.

Major Findings:

Studies on the embryotoxicity in mice of two anthracenediones have been completed. Dihydroxyanthracenedione (NSC-279836) produced significant embryoletality at doses of 5 mg/kg or higher when the compound was injected on day 6 or 7 of gestation, and a parallel increase in the incidence of malformations and a decrease in fetal body weights was noted. The day 8 embryo tolerated a dose of 5 mg/kg, but marked embryotoxicity, manifested as increases in the incidence of embryoletality and malformations and decreases in fetal body weights, was found at doses of 10, 15 and 25 mg/kg. Day 9 and day 10 mouse embryos were resistant to the embryoletal effects of dihydroxyanthracenedione at doses up to 15 mg/kg; however, this dose produced a significant increase in the incidence of malformations and a decrease in average fetal body weights. Regardless of the day

of gestation on which it was administered, dihydroxyanthracenedione produced a significant reduction in maternal body weights at doses of 5 mg/kg or higher. In contrast to dihydroxyanthracenedione, anthracenedione itself was not significantly embryotoxic when administered at 5, 25 or 50 mg/kg to pregnant mice on days 6,7,8,9 or 10 of gestation. In all treatment groups, the incidence of intrauterine deaths, the incidence of malformations, and average fetal body weights resembled controls, although the 50 mg/kg dose produced significant body weight reductions in the pregnant females.

Studies on the embryotoxicity of spirogermanium have also been completed. The compound has been given to pregnant mice as single ip injections on day 6, 7 or 8 of gestation (5,10,20,40 and 80 mg/kg), and as multiple injections (5,10,20 and 40 mg/kg) on days 6-10 of gestation. When given as a single dose on day 6 of gestation, the drug had no significant effect on the incidence of intrauterine deaths, the incidence of fetal malformations, or the average maternal body weight gain during gestation. The only significant effect was related to average fetal body weights, which were higher than controls in the 80 mg/kg group. Following day 7 treatment with spirogermanium, a significant ($p < 0.05$) increase in the incidence of intrauterine deaths was noted in the 40 mg/kg group; however, no such increase in mortality was found in fetuses exposed to the 80 mg/kg dose. Spirogermanium administered on day 8 of gestation had no effect on any of the parameters evaluated, although maternal body weight gains were somewhat reduced in the 10, 20 and 40 mg/kg groups. Similarly, no significant differences in any parameters examined were found between controls and animals exposed on days 6-10 of gestation to spirogermanium at 5, 10, 20 or 40 mg/kg. A total of 5 malformed fetuses was found among the 1005 fetuses examined; the malformations consisted of 2 cases of cleft palate found in control fetuses, 2 fetuses with skeletal abnormalities (spirogermanium, days 5-10, 5 mg/kg; spirogermanium, day 6, 80 mg/kg), and one case of spina bifida in a fetus exposed to spirogermanium (40 mg/kg) on days 6-10 of gestation. Thus it appears that spirogermanium does not have marked embryotoxicity in CD-1 mice under the experimental conditions employed in the present study.

In a recently initiated study, the possibility of preventing drug-induced infertility in males by suppressing spermatogenesis prior to, and during, administration of cyclophosphamide (CTX) or procarbazine (MIH) is being evaluated. Both of these agents are known to induce infertility in human and rodent males, presumably by their non-selective action against rapidly proliferating cells, including spermatogonial stem cells. The rationale of this study is that suppression of spermatogenesis by sex steroids will induce a nonproliferative phase in the primary spermatogonia and that during this phase they will be less vulnerable to the toxic effects of CTX and MIH. Pilot studies identified the level of testosterone in sustained-release capsules required for testicular suppression as measured by serum FSH concentration and prostate weights. Testosterone-containing capsules were implanted 3 weeks prior to initiation of treatment with CTX or MIH; control groups included mice implanted with capsules and receiving saline injections, and mice without capsules injected with CTX or MIH. Drug doses were chosen to correspond to those used in clinical situations and drug treatment was continued for 10 weeks, which corresponds to 2 spermatogenic cycles in the mouse. At the end of the treatment period, the testosterone capsules were removed, and a subset of mice sacrificed for quantification of

serum FSH, LH and testosterone levels and for testicular histology. Additional subsets of animals will be sacrificed for the same purpose at 4 further 8-week intervals. The remainder of the mice will be used in serial mating studies beginning 8 weeks after the end of drug treatment. These mating studies will be carried out to assess fertility in all groups of mice, as well as to determine the possible effects of drug treatment on the survival, frequency of malformations and tumor incidence in their offspring. The segment of the study involving testicular suppression and treatment with CTX and MIH has been completed, and serial mating studies will begin on 5/18/81.

Evaluation of antitumor agents for carcinogenic activity in monkeys

From our survey of second tumors in treated cancer patients, we have accumulated evidence that Hodgkin's disease patients receiving treatment with the MOPP regimen, one component of which is procarbazine, are at increased risk of developing AML. Although a causal relationship between cytotoxic drug therapy and the appearance of AML in these patients has not yet been established, there is ample evidence that procarbazine is a potent carcinogen in mice and rats. In addition, the carcinogenic potential of procarbazine in 3 species of non-human primates has been under study for 15 years. A total of 50 monkeys have survived 6 months or longer after the first dose of drug. Thirteen of the 41 monkeys (26%) necropsied thus far have had malignant neoplasms, seven of which were acute leukemia. Three monkeys developed osteogenic sarcomas, 2 monkeys developed hemangiosarcomas, and a single case of lymphocytic lymphoma was found. The average total dose of procarbazine received by the monkeys developing malignancies was 50.8 gm, the average duration of procarbazine treatment was 86 months. The 9 surviving monkeys in this study may represent a population at high risk for developing AML. For this reason, they are being utilized in hematologic and cytogenetic studies in order to determine whether a "pre-leukemic" phase is detectable prior to the development of frank drug-induced leukemia. For this purpose, sequential bone marrow samples from all of the monkeys receiving procarbazine are being examined for alterations in cellular morphology and for chromosomal aberrations. Since initiation of these studies, no specific chromosomal aberrations have been detected in bone marrow cells from the monkeys and no additional cases of acute leukemia have developed. However, a number of the toxic effects of procarbazine seen clinically are also noted in the monkeys, including vomiting and myelosuppression. Its most striking toxic effect, however, is on the reproductive system of the males. The majority of the adult males necropsied to date have had testicular atrophy with complete aplasia of the germinal epithelium.

There is some evidence that patients receiving long-term treatment with melphalan for multiple myeloma or ovarian cancer may also be at increased risk of developing AML. Although a causal relationship between melphalan treatment and the development of AML in these patients has not yet been established, the carcinogenic activity of this agent has been demonstrated in mice and rats. The carcinogenic potential of melphalan in non-human primates is therefore under study. Twenty monkeys are currently being treated by the oral route with melphalan (1.2 mg/m²) daily, 5 days a week. One group of 10 monkeys has received an average cumulative melphalan dose of 1.32 gm/m² over an average of 55 months. The second group of 10 monkeys has been receiving melphalan for an average of 65

months, and during this time has ingested an average cumulative drug dose of 1.56 gm/m^2 . Women receiving prophylactic melphalan therapy for ovarian carcinoma would receive in the prescribed 18-month dosing period a total melphalan dose of 660 mg/m^2 , a dose lower than that already ingested by these monkeys. None of the monkeys on this study have died and all appear to be healthy.

Kidney transplant recipients and other patients under chronic immunosuppressive therapy with azathioprine appear to be at risk of developing malignancies, primarily lymphomas. Whether this increased risk is related to a direct oncogenic effect of azathioprine or is secondary to a prolonged immunosuppressed state is at present unclear. The carcinogenic potential of azathioprine is being evaluated in two groups of monkeys receiving the drug daily, 5 days every week at doses of 2 and 5 mg/kg, respectively. The 2 mg/kg group is comprised of 14 animals that have thus far received an average cumulative azathioprine dose of 4.38 gm over the course of approximately 38 months. Ten animals are receiving azathioprine at 5 mg/kg, and have ingested an average cumulative dose of 8.06 gm over an average dosing interval of 29 months. None of the monkeys on this study have developed a malignancy and all appear to be healthy.

N-methylnitrosourea (MNU) administered by iv injection has been reported by clinicians in the Soviet Union to be effective in Hodgkin's disease and undifferentiated carcinoma of the lung, and the nitrosoureas BCNU, CCNU and methyl-CCNU have been used in this country to treat a variety of human tumors. The latter 3 agents have been implicated in the development of second tumors in 2 patients receiving treatment for CNS neoplasms. Both patients were diagnosed with acute non-lymphocytic leukemia after receiving oral doses of nitrosoureas totalling approximately 1420 and 2700 mg/m², respectively. We have evaluated the carcinogenic potential of MNU in three species of non-human primates. A total of 43 monkeys have received oral doses of MNU for periods up to 56 months. Nine of the 18 monkeys (50%) necropsied thus far have had squamous cell carcinoma (SCA) of the mouth, pharynx and/or esophagus; upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. The tumors developed in monkeys ingesting cumulative MNU doses averaging 120.0 gm (range 53.2-180.6 gm) over an average of 93 months (range 57-133 months). A minimum of approximately 50 gm of MNU appears necessary for tumor induction by the oral route. Many parallels were noted between the esophageal SCA observed in the present series of monkeys and human esophageal carcinoma, including the clinical manifestations of the tumor, its complications, its radiographic appearance and its morphology. The MNU-induced lesions of the oropharynx and esophagus of primates may therefore be a valuable model for the study of human esophageal carcinoma.

The potential carcinogenicity of adriamycin was also evaluated. A group of 10 monkeys received an iv dose of drug (12 mg/m^2) once each month for 23-27 months. At the end of the dosing period it was intended to hold the animals under observation for the remainder of their lives. However, approximately 2 months after the last dose of adriamycin 8 of 10 animals developed congestive heart failure. Histopathologic examination of cardiac muscle taken at necropsy revealed lesions characteristic of adriamycin-induced cardiomyopathy in humans, and in some cases these findings were confirmed by electron microscopy. In man, a cumulative

adriamycin dose of 550 mg/m^2 has been associated with cardiac toxicity; non-human primates appear to be more sensitive to adriamycin-induced cardiomyopathy, as the monkeys in the present study had received an average cumulative dose of 310 mg/m^2 (range $276\text{--}336 \text{ mg/m}^2$). One of the 10 monkeys developed acute myeloblastic leukemia after receiving 324 mg/m^2 of adriamycin divided into 27 monthly doses. The 10th monkey in this series is alive and without evidence of illness. It received 25 injections of adriamycin totaling 300 mg/m^2 , and received the last dose of adriamycin 38 months ago. This study is being repeated, using 2 groups of 10 monkeys each; the monkeys are receiving monthly iv injections of adriamycin at 2.4 and 4.8 mg/m^2 ; dosing will be terminated when a cumulative dose of 240 mg/m^2 is attained. Thus far, monkeys receiving adriamycin at 2.4 and 4.8 mg/m^2 have been given cumulative drug doses of 69.6 mg/m^2 and 110.4 mg/m^2 , respectively. None of the monkeys have as yet developed signs of congestive heart failure or other indications of ill health.

Cyclophosphamide is widely used as a single agent and in combination regimens for treating human cancer; it is also being used with increasing frequency for treating various non-malignant disorders such as rheumatoid arthritis. The association of transitional cell carcinomas of the urinary bladder and acute myelogenous leukemia with prolonged cyclophosphamide treatment of both malignant and non-malignant disease represents the basis for a recently initiated study on the carcinogenic potential of cyclophosphamide in nonhuman primates. Cyclophosphamide is being administered to a group of 20 monkeys orally, 5 days every week, beginning at 6-7 months of age. The initial dose (3 mg/kg) is increased to 6 mg/kg after 6 months. This study has been underway for an average of only 6 months; during this period an average cumulative cyclophosphamide dose of 0.78 gm has been administered. Three of the monkeys died recently during an outbreak of measles in the colony. Although no gross evidence of tumor was present at autopsy, the histopathology reports for those monkeys are pending.

Significance to Biomedical Research:

Cancer chemotherapeutic agents are in increasing use in the treatment of various non-malignant conditions such as psoriasis, chronic glomerulonephritis and rheumatoid arthritis. Advances in chemotherapy have led to improvements in survival in many types of human cancer. These developments have now made it imperative to evaluate the long-term consequence of exposure to antineoplastic and immunosuppressive agents. For example, there is an increasingly large population of successfully treated (possibly cured) Hodgkins disease patients, and considerable concern has been generated as to their fertility and the effects of the chemotherapy they received on their offspring; subfertility and infertility appears to be a frequent complication of therapy in males. The present study is a first attempt to prevent antitumor agent-induced male sterility. The study will also provide much-needed experimental data pertaining to the potential adverse effects on the offspring of males exposed to antitumor agents prior to mating. The problem of second malignant neoplasms developing in successfully treated cancer patients also appears to be increasing in magnitude. Studies on the carcinogenicity of antineoplastic and immunosuppressive agents in nonhuman primates will aid in identifying those agents with the greatest potential for producing neoplasms in man, and will provide information on the cumulative dose and latent period required for tumor development.

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2. Sieber, S. M., Botkin, C. C., Leslie, K. A., and Cooney, D. A.: Embryo-toxicity in mice of phosphonacetyl-L-aspartic acid (PALA). II. Studies on its mechanism and reversibility. Teratology 22: 321-328, 1980.
3. Adamson, R. H. and Sieber, S. M.: Carcinogenic potential of drugs. Environ. Pathol. Toxicol. 3: 377-385, 1980.
4. Adamson, R. H. and Sieber, S. M.: Studies on the Oncogenicity of Procarbazine and Other Compounds in Nonhuman Primates. In Advances in Malignant Lymphomas: Etiology, Immunology, Pathology and Treatment. 1981 (in press).
5. Adamson, R. H. and Sieber, S. M.: Chemical Carcinogenesis in Nonhuman Primates. In Proceedings of a Workshop on Non-Human Primates in Cancer Research. 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03506-18 LCHP																																								
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COOPERATING UNITS (if any) Radiation Oncology Branch, NCI; and Pediatric Oncology Branch, NCI.																																										
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SUMMARY OF WORK (200 words or less - underline keywords) Studies were continued on the metabolic disposition of <u>misonidazole</u> (NSC-261037), <u>desmethylnisonidazole</u> (NSC-261036), and <u>m-AMSA</u> (NSC-249992). The <u>O-demethylation</u> conversion of <u>misonidazole</u> to <u>desmethylnisonidazole</u> by <u>rat liver microsomes</u> was characterized. An <u>isolated rat liver perfusion system</u> was used to study the dose-dependent <u>elimination kinetics</u> of <u>misonidazole</u> and <u>desmethylnisonidazole</u> . Studies on the <u>clinical pharmacokinetics</u> of <u>desmethylnisonidazole</u> were initiated in conjunction with the Phase I clinical evaluation of this agent. Conditions for the <u>in vitro microsomal metabolism</u> of <u>m-AMSA</u> were established. Studies on the <u>oral absorption</u> and <u>enterohepatic recirculation</u> of <u>m-AMSA</u> were completed. The distribution of <u>m-AMSA</u> in the <u>CSF of monkeys</u> was determined following <u>intraventricular administration</u> and by <u>lumbar puncture</u> .																																										

Project DescriptionObjectives:

To study the metabolic fate of experimental antitumor agents prior to clinical trial and to ascertain whether their metabolites are active in various tumor systems.

To study the pharmacology of antitumor drugs of current interest in clinical chemotherapy in order to design better therapeutic regimens.

To study the clinical pharmacology of new agents in phase I clinical trials.

To evaluate the usefulness of new tumor systems in experimental chemotherapy.

To design new anticancer drugs by rational approaches.

Methods Employed:

Usual pharmacologic, biochemical, analytical, and immunologic techniques.

Major Findings:Dose Dependent Elimination of Misonidazole (NSC-261037) and Desmethylmisonidazole (NSC-261036) in the Isolated Perfused Rat Liver

Clinical trials are now in progress to evaluate the effectiveness of misonidazole and one of its metabolites desmethylmisonidazole as hypoxic cell radiosensitizers. Misonidazole is extensively metabolized in both man and laboratory animals with less than 20% of the unchanged drug appearing in the urine. In patients, the major factor limiting the clinical dosage and ultimate tumor concentration of misonidazole is neurotoxicity. The relationship between the neurotoxicity of misonidazole and its metabolism is not clear; however, in humans we have found that this toxicity is correlated with tissue exposure to misonidazole, as measured by the area under the plasma concentration-time curve. Dose dependent kinetics have been suggested by results of studies in the dog, mouse, and monkey. We have used the isolated perfused rat liver to describe more fully the dose-dependent clearance of misonidazole and to gain insight into the mechanism(s) involved in the clearance of both misonidazole and desmethylmisonidazole by that organ. Our results showed that the simplest model which can accurately describe the desmethylmisonidazole clearance from the perfusate consists of a saturable elimination pathway ($V_{max_3}=32$ nmol/min, $K_{m_3}=11$ μ M) in parallel with a first order pathway ($Cl_5=.21$ ml/min). A similar model was constructed for misonidazole ($V_{max_2}=110$ nmol/min, $K_{m_2}=10$ μ M, $Cl_4=.36$ ml/min) but required an additional saturable pathway ($V_{max_1}=226$ nmol/min, $K_{m_1}=1850$ μ M) to characterize the generation of desmethylmisonidazole, as suggested by our *in vitro* rat liver microsomal studies. A good correlation was demonstrated between desmethylmisonidazole perfusate concentrations (generated during the course of misonidazole perfusions) and simulations based on the misonidazole model which included the microsomal data. The misonidazole and desmethylmisonidazole models demonstrate that the relative contributions of the different pathways for

misonidazole and desmethylmisonidazole elimination are strongly concentration-dependent and that the misonidazole $\rightarrow$ desmethylmisonidazole pathway is a minor route. A qualitative similarity in saturation kinetics was observed in the disappearance curves for both misonidazole and desmethylmisonidazole from the liver perfusate. When misonidazole was combined with an excess of desmethylmisonidazole, a marked decrease in the clearance rate of misonidazole from the liver perfusate was noted. This observation suggests that misonidazole and desmethylmisonidazole are metabolized along similar routes. Further studies are being undertaken to establish the relationship between the proposed model for misonidazole and desmethylmisonidazole elimination as developed from results with the isolated perfused liver system and the dose dependent kinetics observed *in vivo*; we are also investigating the possibility that misonidazole at high doses is preferentially converted to a toxic metabolite and that this may be a factor in its neurotoxicity.

Studies on the O-Demethylation of Misonidazole (NSC-261037) By Rat Liver Microsomes

Desmethylmisonidazole (NSC-261037) has been identified as a metabolite of misonidazole. Both misonidazole and desmethylmisonidazole are effective hypoxic cell radiosensitizers. The role of rat liver microsomes in the O-demethylation of misonidazole to desmethylmisonidazole was studied. The rate of microsome-dependent formation of desmethylmisonidazole was linear up to a protein concentration of 2 mg/ml and over a 10 min time interval. The metabolism was optimal in a system comprised of microsomes, oxygen, and NADPH. When the incubation mixtures were continuously flushed with CO or N₂, the metabolism was inhibited by 83 and 78%, respectively. The addition of SKF 525-A resulted in competitive inhibition with a K_i of approximately 1×10^{-5} M. The K_m and V_{max} values of normal microsomes were 1.87 ± 0.30 mM and 413 ± 14 pmoles/min/mg protein, respectively. Pretreatment of rats with phenobarbital for 7 days prior to preparation of the microsomes resulted in no significant change in the K_m, but the V_{max} was considerably increased to 1033 ± 203 pmoles/min/mg protein. The results indicate that the O-demethylation of misonidazole is mediated by a cytochrome P-450 mixed function oxidase.

Pharmacokinetics of Desmethylmisonidazole (NSC-261036) in Man

Clinical studies with misonidazole have indicated that the dose limiting toxicity associated with this drug is neurotoxicity. Previous clinical studies at NCI with intravenous administration of misonidazole have shown a strong correlation between the extent of neurotoxicity and the area under the plasma misonidazole concentration-time curve. This is in agreement with results reported by other investigators following oral administration of misonidazole. Desmethylmisonidazole, a metabolite of misonidazole, in animal systems yields X-ray dose enhancement ratios similar to misonidazole and may possess pharmacological advantages over the parent compound. A comparison of the pharmacokinetics of misonidazole and desmethylmisonidazole in animals showed that the latter compound has a reduced area under the plasma concentration-time curve, a shorter plasma elimination half life, and increased urinary excretion. Based on these observations, clinical trials of desmethylmisonidazole have been initiated. To date, two patients at NCI have been entered into the study. The pharmacology has been investigated following doses of 1 g/m^2 and 2 g/m^2 . The

results of the analysis showed an initial mean distribution half time of 9 min $\pm$ 6 min SD and a terminal plasma elimination half life of 6.1 hrs $\pm$.5 hr SD. Renal excretion of desmethylmisonidazole was 51% and 50% of the total dose administered, respectively, in the two subjects. An apparent volume of distribution (mean = 766 ml/kg $\pm$ 100 ml/kg) was determined. These data indicate that, as observed in animals, desmethylmisonidazole has a shorter elimination half time and higher renal excretion than does misonidazole and may offer pharmacological advantages over the parent compound as a radiosensitizer.

Metabolism of m-AMSA (NSC-249992) by Rat Liver Microsomes

Studies on the metabolism of m-AMSA by isolated rat liver microsomes were continued. Metabolism was optimal in a system comprised of microsomes, oxygen, cytosol, and a NADPH generating system. Metabolism was decreased by approximately 90% when either the NADPH generating system, microsomes, or cytosol were omitted from the incubation mixture and by 90% when both microsomes and the generator were omitted. Flushing the incubation mixtures continuously with CO, which inhibits cytochrome P-450, or with N₂, which removes the required oxygen, also markedly inhibited this metabolism. α -Naphthoflavone, metyrapone, and SKF 525-A, all inhibitors of hepatic microsomal-mediated drug oxidation, reduced the metabolism by 77%, 75%, and 65%, respectively. The metabolism was markedly enhanced with microsomes prepared from rats pretreated with phenobarbital or 3-methylcholanthrene. By 10 mins after the initiation of the incubation, 5.1 and 13.05 nmoles of m-AMSA were metabolized by phenobarbital and 3-methylcholanthrene microsomes, respectively, as compared to 2.1 nmoles by normal microsomes. The apparent K_m obtained with normal or phenobarbital microsomes was similar (5.0×10^{-6} M and 7.7×10^{-6} M, respectively), while the K_m obtained with 3-methylcholanthrene microsomes was approximately 10 fold greater (7.6×10^{-5} M). The V_{max} with normal microsomes was 7.1 nmoles/2 mg/15 min as compared to a 2.5 fold increase with phenobarbital microsomes (17.6 nmoles/2 mg/15 min), and a 30 fold increase (220.1 nmoles/2 mg/15 min) with 3-methylcholanthrene microsomes. Previously, two quinoidal intermediates, m-AQDI and m-AQI, were detected upon HPLC analysis of the ethyl acetate extract of microsomal incubations. Since m-AQDI can be formed via a N-hydroxylated intermediate, the metabolism of m-AMSA is postulated to proceed via this route, resulting in bioactivation to a highly reactive species in vivo.

Oral Absorption of m-AMSA (NSC-249992) and Possible Enterohepatic Recirculation

Although m-AMSA has routinely been administered to patients by the intravenous route, it has been given orally as well. Previous work in our laboratory showed that plasma concentrations of m-AMSA were much lower for oral versus comparable intravenous administration. This difference could be explained by poor gut absorption of drug, rapid clearance of drug from the portal system, or both. We undertook studies to evaluate these various possibilities.

In Sprague-Dawley rats, 80% of m-AMSA was absorbed from the gut in 8 hours when a dose of 200 mg/kg was administered. Higher doses were not studied because a dose of 250 mg/kg was uniformly lethal. At 25 mg/kg the 8 hour absorption was over 90%. More detailed studies, utilizing isolated segments of jejunum and ileum, revealed that solubility might be a limiting factor in the absorption of the drug. Segments of jejunum or ileum were ligated to create intestinal sacs

approximately 12 cm in length. These sacs were filled to mild distension with ^{14}C -labeled m-AMSA in physiologic saline at a concentration of $2.5 \times 10^{-5} \text{ M}$. Maximum solubility under these conditions is $5.5 \times 10^{-5} \text{ M}$. Within 15 minutes, 50% of the m-AMSA was absorbed and 90% was absorbed in 1 hour. Since an oral dose of 200 mg/kg of m-AMSA would require a volume of 4-6 liters of intestinal fluids to completely dissolve, oral administration of drug must expose the intestinal microvilli to an m-AMSA concentration that is close to saturation. As drug is absorbed, more drug dissolves and a quasi-saturated condition is maintained.

Since m-AMSA is rather completely absorbed, the low plasma levels must be caused by rapid removal of drug by the liver. This rapid hepatic extraction gives rise to the possibility of enterohepatic recirculation of parent drug or of metabolite. This possibility was addressed through a dual rat experiment in which the bile outflow of one rat was directed into the duodenum of a second rat. The first rat was given ^{14}C -labeled m-AMSA and absorption of radioactivity into the second rat was measured. Four experiments were performed and the second rat absorbed $9.3 \pm 5.6\%$ of the available radioactivity. These data indicate that some enterohepatic recirculation of m-AMSA and its metabolites does occur, but the proportion of drug recirculated is small and probably clinically insignificant.

Distribution of m-AMSA (NSC-24992) in the CSF of the Rhesus Monkey

m-AMSA has been used with considerable clinical success against the leukemias, with complete remissions reaching 30% in previously treated patients. Because of its anti-leukemic activity, m-AMSA is being considered as a third-line drug against meningeal leukemia which is refractory to standard therapy. Previous work has shown that m-AMSA crosses the blood-brain barrier with difficulty. Intraventricular administration of 50 μg m-AMSA showed a probable biphasic clearance from the CSF with peak concentrations of $2.4 \times 10^{-4} \text{ M}$. When the administered dose was increased to 500 μg , the peak concentration was increased tenfold to $2.3 \times 10^{-4} \text{ M}$. At neither dose, 50 μg or 500 μg , did the monkeys exhibit significant neurotoxicity. m-AMSA was also administered (500 μg in 0.8 ml of steroid suspending vehicle) by lumbar puncture and samplings were made from the 4th ventricle through an Ommaya reservoir. These studies are not quite complete but, so far, drug has not been detected in the ventricular fluid. If the drug can not enter ventricular fluid following lumbar puncture, its use in meningeal disease will be restricted to patients with Ommaya reservoirs in place.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03509-18 LCHP															
PERIOD COVERED October 1, 1980 to September 30, 1981																	
TITLE OF PROJECT (80 characters or less) Carcinogenesis, Chemotherapy and Biological Markers in Non-Human Primates																	
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																	
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Richard H. Adamson</td> <td style="width: 33%;">Chief, Lab. Chem. Pharm.</td> <td style="width: 33%;">LCHP NCI</td> </tr> <tr> <td>Other: Susan M. Sieber-Fabro</td> <td>Head, Pharm. & Exp. Ther. Sec.</td> <td>LCHP NCI</td> </tr> <tr> <td>K. Robert McIntire</td> <td>Chief, Diagnosis Branch</td> <td>DB NCI</td> </tr> <tr> <td>Pelayo Correa</td> <td>Pathologist</td> <td>Louisiana State University</td> </tr> <tr> <td>Dan W. Dalgard</td> <td>Veterinarian</td> <td>Hazleton Laboratories</td> </tr> </table>			PI: Richard H. Adamson	Chief, Lab. Chem. Pharm.	LCHP NCI	Other: Susan M. Sieber-Fabro	Head, Pharm. & Exp. Ther. Sec.	LCHP NCI	K. Robert McIntire	Chief, Diagnosis Branch	DB NCI	Pelayo Correa	Pathologist	Louisiana State University	Dan W. Dalgard	Veterinarian	Hazleton Laboratories
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																	
SUMMARY OF WORK (200 words or less - underline keywords) Twenty-seven substances, including antitumor agents, contaminants of human food-stuffs, rodent carcinogens, pesticides, and artificial sweeteners are being evaluated in four species of <u>non-human primates</u> for their potential carcinogenicity and other long-term toxic effects. Sixteen of these substances have not as yet demonstrated carcinogenic activity, although some have been on test for less than 2 years. Eight of the compounds are carcinogenic in non-human primates, producing tumors in 0.1-100% of the treated animals. <u>1-methyl-1-nitrosoourea</u> induced squamous cell carcinomas of the oropharynx and esophagus, with the esophageal tumors possessing clinical and morphologic similarities to human esophageal carcinoma. Long-term treatment with <u>procarbazine</u> resulted in an increased incidence of malignancies, approximately one-half of which were leukemias. The effects of 6 of the 8 compounds (<u>DENA</u> , <u>DPNA</u> , <u>1-nitrosopiperidine</u> , <u>aflatoxin B₁</u> , <u>MAM-acetate</u> and <u>urethane</u>) were manifested primarily as hepatocarcinogenicity. Single cases of malignant tumors have been diagnosed in animals treated with <u>adriamycin</u> , <u>butter yellow</u> , and <u>N-methyl-N'-nitro-N-nitrosoguanidine</u> . Monkeys bearing <u>DENA</u> -induced hepatomas were used to develop a new <u>lipoid-based contrast material (EOE 13)</u> for computed tomography of liver and spleen.																	

Project Description

Objectives:

To evaluate the carcinogenic potential and long-term adverse effects of clinically useful antineoplastic and immunosuppressive agents.

To obtain data on which to base a comparison of the response of non-human primates and rodents to materials known or suspected to be carcinogenic to man, and thereby to evaluate the relative merits of rodents and non-human primates in predicting for man.

To develop model tumor systems in primates for evaluating the potential usefulness of new anticancer agents active against rodent tumors before these agents are administered to cancer patients.

To determine whether the non-human primate resembles the rodent in that it is more susceptible to chemical carcinogenesis as a neonate than as an adult.

To evaluate the possibility of preventing or reversing chemical carcinogenesis in non-human primates with nucleophiles or immunostimulation.

To use normal and tumor-bearing non-human primates for studying the pharmacological, toxicological, and chemotherapeutic properties of various anticancer, antiviral, and antimicrobial agents.

To carry out comparative biochemical and immunological studies using normal animals, animals with preneoplastic lesions, and animals with malignant neoplasms.

To develop methods for detecting preneoplastic changes and accomplishing the early diagnosis of tumors.

Methods Employed:

Compounds Under Investigation:

Twenty-seven substances are currently under investigation or have been investigated, including: 3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, ethyl carbamate, N,N'-dimethyl-p-phenylazo-aniline, N,N'-dimethyl-p-(m-tolylazo)-aniline, methylnitrosourea, aflatoxin B₁, methylazoxymethanol acetate, cyclamate, saccharin, dichlorodiphenyltrichloroethane, N-methyl-N'-nitro-N-nitrosoguanidine, low density polyethylene plastic, cigarette tobacco smoke condensate, 1-nitrosopiperidine, N-nitrosodiethylamine, N-nitrosodipropylamine, arsenic, sterigmatocystin, Imuran, Adriamycin, procarbazine, L-phenylalanine mustard and cyclophosphamide.

The compounds are administered subcutaneously, intravenously, intraperitoneally or orally. For oral administration to newborn monkeys, the compound is added to the Similac formula at the time of feeding; when the monkeys are six months old, carcinogens given orally are incorporated into a vitamin mixture which is given to monkeys as a vitamin sandwich on a half slice of bread. An alternate way of giving doses orally is to incorporate the compound into a prune or to intubate. The dose level chosen is dependent on the chemical under evaluation. Antineoplastic and immunosuppressive agents are administered at doses likely to be encountered in a clinical situation; other substances, such as environmental contaminants, are given at levels 10-40 fold higher than the estimated human exposure level. The remainder of the chemicals tested are administered at maximally tolerated doses which, on the basis of weight gain, blood chemistry and hematology findings, and clinical observations, appear to be devoid of acute toxicity.

Animals

The present colony, consisting of approximately 500 animals, is comprised of four species: Macaca mulatta (rhesus), Macaca fascicularis (cynomolgus), Cercopithecus aethiops (African green) and Galago crassicaudatus (bushbabies). Fifty-four of these monkeys are adult breeders which until recently supplied the newborns for experimental studies. The majority of the animals are housed in an isolated facility which contains only animals committed to this study, and with the exception of the breeding colony, most animals are housed in individual cages. The administration of test compounds is usually initiated within 24 hours of birth and continues until a tumor is diagnosed or until a predetermined exposure period has been completed. A minimum of 30 animals is usually allotted to each treatment group, since in a sample of this size it is possible to detect a tumor incidence of 10% within 95% confidence limits.

A variety of clinical, biochemical and hematological parameters are monitored weekly or monthly, not only to evaluate the general health status of each animal, but also for the early detection of tumors. Surgical procedures are performed under phenacylidine hydrochloride, Ketamine or sodium pentobarbital anesthesia. All animals which die or are sacrificed are carefully necropsied and the tissues subjected to histopathologic examination.

Major Findings:

Compounds Carcinogenic in Non-Human Primates

Eight of the 27 substances evaluated have induced malignant neoplasms in non-human primates, producing a tumor incidence ranging from 9.1-100% of the treated animals. The compounds are: N-nitrosodiethylamine, 1-nitrosopiperidine, N-nitrosodipropylamine, aflatoxin B₁, methylazoxymethanol acetate, procarbazine, methyl nitrosourea, and urethane. In addition, single cases of malignant tumors have been diagnosed in animals treated with adriamycin, N-methyl-N'-nitro-N-nitrosoguanidine and N,N'-dimethyl-p-phenylazoaniline (butter yellow).

Nitrosoamine Carcinogenesis

N-nitrosodiethylamine (DENA) has induced tumors in 33 out of 45 Old World monkeys receiving oral treatment with 40 mg/kg 5 times a week. These tumors, all of which were hepatocellular carcinomas, developed in rhesus, cynomolgus and African green monkeys which had received the initial dose of DENA either at birth, at 1-8 months postpartum, or as adults. There is some indication that monkeys receiving the initial dose of DENA at birth required less total DENA for tumor development than those monkeys in which initial treatment was delayed until 1-8 months postpartum or until adulthood. However, the number of animals in each group is not sufficiently large to test the statistical significance of this apparent difference. An apparent species difference exists with respect to both latent period and cumulative dose for tumor induction. The latent period averaged 26, 49 and 105 months for cynomolgus, rhesus and African green monkeys, respectively. The average total DENA dose ingested by cynomolgus monkeys developing tumor was 18.0 gm; for rhesus monkeys this value was 25.4 gm, and for African greens it was 55.1 gm. This apparent species difference was not noted, however, when DENA was given by the ip route. A total of 106 cynomolgus, rhesus, African green and rhesus x cynomolgus hybrid monkeys given bimonthly doses (40 mg/kg) of DENA developed hepatocellular carcinomas. The latent period averaged for the 4 species was 17 months (range 15-17 months) and the average total dose of DENA necessary for tumor development was 1.7 grams (range 1.32-1.95 gm). The latent period and the total dose required for tumor induction by the ip route appeared to be independent of the age at which dosing was initiated.

DENA is also carcinogenic in the more primitive primate Galago crassicaudatus. All 10 treated animals have developed tumors after bimonthly ip injections of DENA at doses of 10-30 mg/kg. In contrast to the DENA-induced primary hepatocellular carcinomas in Old World monkeys, all 10 of the bushbabies developed mucoepidermoid carcinomas of the nasal cavity. In 2 of these 10 animals, carcinoma of the liver was also present, and in both cases metastases to the lungs or to intestinal lymph nodes was noted. The average total dose of DENA given the bushbabies was 0.747 gm, and ranged from 0.295-1.485 gm. The latent period averaged 20 months (range 12-27 months).

Two other nitrosamines have induced primary hepatocellular carcinomas in monkeys. N-nitrosodipropylamine (DPNA) induced liver tumors in all 6 of the rhesus and cynomolgus monkeys given bimonthly ip doses of 40 mg/kg. The average total dose of DPNA was 7.0 gm; the average latent period for tumor development was 28.5 months. 1-nitrosopiperidine (PIP) is also an hepatocarcinogen in macaques. Hepatic cell carcinomas developed in 11 of 12 monkeys receiving this compound by the oral route, and in 5 out of 11 monkeys treated by the ip route. The average cumulative dose necessary for tumor induction by PIP given orally (1742.5 gm) was higher than for oral DENA (18.0-55.1 gm); similarly, the average cumulative dose of PIP given by the ip route (39.4 gm) exceeded that required for tumor induction by ip DENA (1.7 gm) or ip DPNA (7.0 gm).

A pediatric laparoscope has recently been acquired and employed in monkeys receiving treatment with DENA and other hepatocarcinogens. Laparoscopy is a rapid, relatively non-invasive procedure; it will make possible the early detec-

tion of tumors, and will enable us to follow their growth and response to chemo- and immuno-therapy.

Carcinogenic Activity of Aflatoxin B₁ and Methylazoxymethanol Acetate

Aflatoxin B₁ (AFB₁) and methylazoxymethanol acetate (MAM-acetate), two substances known to be contaminants of human foodstuffs, are carcinogenic in non-human primates. The carcinogenicity of AFB₁ has been under evaluation in non-human primates for the past 15 years. A total of 47 Old World monkeys, chiefly rhesus and cynomolgus, have received AFB₁ by ip (0.125-0.25 mg/kg) and/or oral (0.1-0.8 mg/kg) routes for 2 months or longer, and 5 are currently alive and without evidence of tumor. Eighteen of the 41 monkeys necropsied to date developed a total of 22 malignant neoplasms, yielding an overall tumor incidence of 38%. Seven of the 18 tumor bearing monkeys developed hemangioendothelial sarcomas of the liver, 5 developed bile duct or gallbladder adenocarcinomas, and 2 cases of hepatocellular carcinoma were diagnosed. One monkey developed an osteosarcoma and 3 were found at necropsy to have multiple primary tumors. All of the latter animals possessed adenocarcinoma of the pancreas as well as urinary bladder carcinoma, adenocarcinoma of the bile ducts and osteosarcoma. The tumors diagnosed in the 18 monkeys developed after an average latent period of approximately 10 years (range 5-12 years) and after an average cumulative AFB₁ dose of 820 mg (range 292-1103 mg). An additional monkey has recently been necropsied and gross evidence of tumor found; although the histopathology report is pending, a tentative diagnosis of fibrosarcoma has been made. Fifteen of the 23 (65%) necropsied monkeys without tumor showed histologic evidence of liver damage, including toxic hepatitis, cirrhosis and hyperplastic liver nodules. Our results indicate that AFB₁ is a potent hepatotoxin and carcinogen in non-human primates, and further support the hypothesis that humans exposed to this substance may be at risk of developing liver cancer.

Cycasin, the active principle in the cycad nut, induces liver and kidney tumors in rats and may be a human carcinogen as well. The carcinogenic potential of cycasin and its aglycone, MAM-acetate, is under investigation in non-human primates. Old World monkeys (rhesus, cynomolgus, and African greens) received cycasin and/or MAM-acetate by po or ip routes for periods up to 12 years. Eighteen monkeys survived > 2 mo after initiation of treatment with cycasin (50-75 mg/kg) or MAM-acetate (1.5-3.0 mg/kg) given po daily 5 days/week, and 11 of the animals have been necropsied. Histopathologic examination of tissue from one of these monkeys revealed hepatocellular carcinoma. A second monkey was noted to have multiple tumors, including hepatocellular carcinoma, intra-hepatic bile duct adenocarcinoma, renal carcinoma and adenomas, and adenomatous polyps of the colon. An adenocarcinoma of the pancreas was diagnosed in a third monkey. Although liver tumors were not observed in the other monkeys, all but one had hepatic lesions such as toxic hepatitis and cirrhosis. A group of 10 monkeys received MAM-acetate by weekly ip injections (3-10 mg/kg). Six of these animals developed tumors after receiving an average of 6.14 gm (range 3.58-9.66 gm) of MAM-acetate for an average of 75 mo (range 50-89 mo). Four of the monkeys developed hepatocellular carcinomas and 2 had multiple primary tumors including hepatocellular carcinomas, renal carcinomas, squamous cell carcinomas of the esophagus and adenocarcinomas of the small intestine. Our results show that MAM-acetate is a carcinogen in monkeys, and add to the evidence that cycasin and

its aglycone may be carcinogenic in man.

Carcinogenic Activity of Methylnitrosourea

Squamous cell carcinoma of the mouth, pharynx, and esophagus developed in 9 of 43 monkeys (21%) receiving oral doses (10-20 mg/kg) of 1-methyl-1-nitrosourea (MNU). Moreover, upper digestive tract lesions such as atrophy or dyskeratosis of the esophageal mucosa and esophagitis have been a consistent finding among the 18 monkeys necropsied to date. All monkeys except one that have received total doses of MNU exceeding 50 grams have developed carcinomas, whereas no malignant tumors have developed in monkeys receiving a cumulative dose less than 50 grams. The average latent period for tumor development was 93 months and ranged between 57-133 months. The order of appearance of the esophageal lesions in our monkeys, as well as the clinical manifestations of the tumors resembled those seen in humans, and included difficulty in swallowing, frequent vomiting and subsequent weight loss, and sialorrhea. The common complications of esophageal carcinoma in humans (e.g., regurgitation, aspiration, sepsis and hemorrhage) were also noted in our monkeys. In addition, histological examination revealed a morphology similar to that seen in human esophageal carcinomas, despite the highly variable nature of such tumors in both human and monkeys. Thus, MNU-induced esophageal carcinoma in non-human primates may prove to be a valuable model for the study of the human tumor.

Carcinogenic Activity of Procarbazine

Fifty monkeys have survived for longer than 6 months after receiving treatment with procarbazine by sc, ip and/or oral routes at doses of 5-50 mg/kg. Forty-one monkeys have been necropsied to date, of which 13 (26%) have had malignant neoplasms. Seven monkeys were diagnosed with acute leukemia, all but one of the myelogenous type; the other acute leukemia was undifferentiated. In addition to the leukemias, two cases of hemangiosarcoma, three cases of osteogenic sarcoma, and one case of lymphocytic lymphoma, was found. The neoplasms were diagnosed after procarbazine treatment for an average of 86 months (ranging from 16 to 148 months); the total dose of procarbazine received by the monkeys developing tumors ranged from 2.69-154.4 gm, and averaged 50.8 gm. Other adverse effects of long-term procarbazine treatment included vomiting, myelosuppression and testicular atrophy with complete aplasia of the germinal epithelium.

Carcinogenic Activity of Urethane

The carcinogenicity of urethane, with or without whole body irradiation (WBI) was evaluated in rhesus and cynomolgus monkeys. Monkeys received urethane (250 mg/kg) orally, 5 days every week beginning within one month of birth. They received continuous urethane treatment for 5 years, during which time some monkeys also received 3-10 weekly courses of WBI at 50 rads per course. Urethane administration was discontinued 11-14 years ago and since that time all animals have been held under close observation for development of tumor or other adverse effects of treatment. Thirty of a total of 40 monkeys survived 6 months or longer after the first dose of urethane, and 22 of these animals have been necropsied. A total of 8 malignant tumors were found in 5 (16.7%) of the 30 treated monkeys; in comparison, 7 of a total of 219 (3.2%) control monkeys have

developed tumors during this period. One or more primary liver tumors (3 cases of hemangiosarcomas, 1 case of adenocarcinoma of intrahepatic bile ducts, 1 case of hepatocellular carcinoma) were present in 4 monkeys; one of the monkeys with a liver hemangiosarcoma was also found to have an ependyoblastoma, and the fifth monkey developed a pulmonary adenocarcinoma. The animals with tumor had received an average cumulative urethane dose of 260 gm (range 230-339 gm); the latent period for tumor induction averaged 171 months (range 142-229 months). Two of the 5 monkeys developing tumors had received 9 and 10 courses of WBI, respectively. These results indicate that urethane, with or without WBI, is carcinogenic in monkeys; however, the latent period for tumor induction (> 14 years) is long, requiring approximately 50% of the usual lifespan of Old World monkeys in captivity.

Potential Carcinogenicity of Adriamycin

The carcinogenic potential of adriamycin is less clear-cut than that of the preceding compounds. A group of 10 monkeys received monthly iv doses of adriamycin at 12 mg/m². Eight of 10 animals developed congestive heart failure before the end of the anticipated dosing period, and at cumulative adriamycin doses (averaging 310 mg/m²) well below that considered to be the safe upper limit (550 mg/m²) in man. The animals died or were sacrificed in moribund condition. One of the 10 monkeys developed acute myeloblastic leukemia after receiving a cumulative adriamycin dose of 324 mg/m², and the 10th monkey in this group is alive and without evidence of illness. Old World monkeys appear to be more sensitive than humans to the cardiotoxic effects of adriamycin, and this may apply to its carcinogenic effects as well. Although the single case of leukemia noted in this study does not establish that adriamycin is a leukemogen, it does suggest that this may be yet another adverse effect of adriamycin therapy. This study is being repeated, using lower doses of adriamycin in an additional twenty monkeys.

Potential Carcinogenicity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)

MNNG is being administered by the oral route (0.4 mg/kg, daily 5 days every week). A group of 21 monkeys has received this compound for periods of up to 8 years; thus far, 2 animals have died of causes unrelated to treatment with MNNG. The remaining 19 animals appear to be in good health and without signs of toxicity. However 3 additional monkeys were given MNNG as a colon implant; 2 monkeys have been necropsied and one monkey was diagnosed with a well-differentiated adenocarcinoma at the rectosigmoid junction. The latter monkey had received a total MNNG dose of 8.65 gm; it was administered in gelatin cubes containing 5.3-42.7 mg MNNG which were inserted into the colon twice every week.

Potential Carcinogenicity of N,N-dimethyl-p-phenylazoaniline (butter yellow)

A total of 32 animals received oral doses of butter yellow, and 24 are alive 18-20 years after the first dose of the compound. Four of the 8 dead animals survived less than 1 year after initiation of treatment; among the 4 necropsied animals that survived longer than one year, one case with a highly invasive liposarcoma was found. Whether this tumor arose as a consequence of treatment with butter yellow or is a spontaneous tumor developing in an aged (20 year)

animal is uncertain; this judgement must await further observation of the 24 surviving monkeys on this study.

Compounds Not Carcinogenic in Non-Human Primates

Since the inception of this study 20 years ago, 7 spontaneous tumors have been diagnosed in 219 non-treated breeders and vehicle-treated controls, yielding a tumor incidence of 3.2%. Of the 27 substances entered on test during this period, 16 [3-methylcholanthrene, dibenz(a,h)anthracene, 3,4,9,10-dibenzpyrene, N-2-fluorenylacetamide, N,N-2,7-fluorenylenebisacetamide, N,N'-dimethyl-p-(π -tolylazo)-aniline, cyclamate, saccharin, dichlorodiphenyltrichloroethane, low density polyethylene plastic, cigarette smoke condensate, arsenic, sterigmatocystin, Imuran, L-phenylalanine mustard and cyclophosphamide] have not as yet induced tumors. However, several of these compounds have been under evaluation for less than 2 years.

Cyclamate has been under test for the past 10½ years. Two groups of monkeys have received this compound orally, 5 days every week, at 100 and 500 mg/kg, respectively. The 100 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 2.3 gm/day/70 kg man, and is equivalent to drinking about 6 diet drinks per day. The 500 mg/kg dose in monkeys corresponds, on an equivalent surface area basis, to a daily intake of 11.6 gm/day/70 kg man, and is equivalent to drinking about 30 diet drinks per day. Two of 12 monkeys at the low dose, and 2 of 11 monkeys at the high dose have been necropsied, but no evidence of a malignant neoplasm was found.

Two groups of 10 monkeys each have been receiving oral doses of saccharin (25 mg/kg), 5 days every week. This dose corresponds, on an equivalent surface area basis, to a daily intake of 5 cans of diet soda by a 70 kg man. One group of monkeys has been receiving saccharin for an average of 122 months (range 120-124 months), and the second group of 10 monkeys began saccharin treatment approximately 3 years ago. Since the inception of the study, none of the monkeys have died, and there is no evidence of toxicity in any of the treated animals.

Similarly, long-term administration of DDT has not resulted in the development of tumors in our nonhuman primates. A total of 15 animals have received DDT by the oral route (20 mg/kg) daily, 5 days every week in a study that has been underway for the past 134 months. Administration of DDT is discontinued after a dosing interval of 130 months is completed. Although 5 of the monkeys have died thus far, none were found to have developed tumor. The apparent cause of death in these animals was DDT-induced CNS toxicity, as all experienced severe tremors and convulsions immediately prior to death. The 10 surviving monkeys appear to be in good health.

DENA Dose-Response Studies and Attempts at Chemoprevention

DENA is highly predictable as a hepatocarcinogen in Old World monkeys, and accordingly we have accumulated a relatively large amount of information on its carcinogenic effects in these animals. We are therefore currently using this chemical as a model carcinogen to investigate two questions of importance to chemical carcinogenesis.

The first question pertains to whether there is a specific total dose of carcinogen which, within the lifespan of the test animal, will induce a tumor. In order to evaluate this question, groups of monkeys are being given bimonthly ip injections of DENA at doses of 0.1, 1, 5, 10, 20 and 40 mg/kg, and are observed for the appearance of tumor. In the 4 groups of monkeys (40, 20, 10 and 5 mg/kg doses) in which tumors have developed, we have found that the latent period increases as the mg/kg dose decreases. Thus with the 40 mg/kg dose the latent period is 17 months, whereas at 5 mg/kg it is 65 months. The study is as yet incomplete and in some groups the proportion of tumor-bearing animals is small, so that it is not yet possible to report a precise value for the carcinogenic dose of DENA. However, it appears that this value will lie between 1.4 and 3.1 gm.

The second question pertains to whether chemically-induced cancer can be prevented or reversed by other chemicals, either administered simultaneously with the carcinogen, or before carcinogen exposure. It is now recognized that the reactive forms of most chemical carcinogens are strong electrophiles and are capable of attacking tissue nucleophiles. Thus, one of the possible approaches to the prevention or reversal of chemical carcinogenesis is to supply an excess of non-critical nucleophiles, thereby "scavenging" the reactive electrophilic species of carcinogens once formed. We are using this approach in 2 groups of monkeys receiving bimonthly ip doses of DENA at 10 mg/kg or 5 mg/kg. In both groups, the DENA dose is given 10 minutes after an ip injection of a "protective cocktail". For the monkeys receiving DENA at 10 mg/kg, the "protective cocktail" consists of l-cysteine (20 mg/kg), cysteamine (20 mg/kg) and reduced glutathione (150 mg/kg). The "protective cocktail" given the monkeys receiving DENA at 5 mg/kg is composed of N-acetylcysteine (50 mg/kg). These substances were chosen as nucleophilic chemoprotectants for two reasons. First, they do not reduce the antitumor effects of various clinically useful alkylating agents and therefore could be used to protect against the carcinogenicity of these drugs without interfering with their therapeutic effects. Secondly, both N-acetylcysteine and cysteamine have been shown to prevent the covalent binding of acetaminophen to tissue, and for this reason have been used in humans to reverse the hepatotoxic effects of acute acetaminophen poisoning. By analogy, it is hoped that these sulfhydryl reagents will prevent the covalent binding of the activated form of DENA to critical macromolecules of liver tissue, an event which is thought to be a prerequisite for hepatocarcinogenesis by a variety of chemicals including DENA. However, during the course of studies with both "protective cocktails", it became apparent that they were producing a significant degree of peritoneal irritation; necropsy of several of the animals revealed intestinal perforation or obstruction attributable to multiple peritoneal abscesses and adhesions. For this reason, dosing with DENA and the "protective cocktails" was discontinued. In the interim, 8 of 10 monkeys receiving DENA (10 mg/kg) and the "protective cocktail" have developed tumor. When dosing with DENA (5 mg/kg) is resumed, N-acetylcysteine will be given by the oral route rather than ip.

Experimental Evaluation of a New Contrast Medium for Computed Tomographic Examination of the Liver and Spleen

A lipid based contrast material (EOE 13) containing 53% of ethiodized oil in emulsion form was developed for computed tomography (CT) of the liver and spleen

and tested in monkeys and other animals. An intravenous dose of 0.2 ml/kg selectively opacified the liver and spleen, resulting in an average increase of 23 EMI units (500 scale) in the attenuation of the liver and a higher increase in the attenuation of the spleen. When injected into rhesus monkeys with carcinogen induced hepatocellular carcinoma, there was a significant improvement in the visualization of the tumor, and small lesions, undetectable on the preliminary CT scan, became visible. Toxicity studies have been completed in animals and the contrast material is in clinical trials at the Clinical Center.

Ten patients with disseminated cancer were given intravenous injections of 0.2 ml/kg (40 mg I/kg) of EOE 13. CT scans of the liver and spleen were taken prior to and 30 minutes after contrast infusion. Visualization of the liver was significantly improved in 5, moderately improved in 3, and not appreciably improved in 2. The spleen showed an obvious increase in density in all cases. No significant toxicity was encountered: untoward side effects consisted of fever, headaches, foul metallic taste, and weakness for a short period. Four patients had no side effects, and 2 experienced only abnormal taste sensation. Further experimental and clinical work is needed before the advantages and safety of this contrast material can be documented.

Significance to Biomedical Research:

The present colony has been in continued existence for 20 years; it supports the largest study of chemical carcinogenesis in nonhuman primates undertaken in this country, and as such it represents a national resource. In addition to providing data on the carcinogenicity of a variety of chemicals, including anti-tumor and immunosuppressive agents in chemical use, it has also made it possible for us to acquire information in other important areas of primatology. Such information includes the spontaneous tumor incidence in various species of nonhuman primates, their lifespan in captivity, their reproductive characteristics, the organization and management required to hand-rear 40-50 neonates per year in a nursery, and parameters of growth and development in simian primates. Normal animals of all ages as well as tumor-bearing animals are used in a variety of pharmacologic studies and chemotherapeutic trials. The availability of monkeys receiving chronic treatment with chemicals has made it possible to identify some consequences of long term exposures (other than tumor development) not previously recognized. Tumor-bearing monkeys have been employed in attempts to develop biological markers, such as α -fetoprotein, and other diagnostic tests for detecting premalignant lesions and early tumors. More recently, tumor bearing monkeys have been used to develop new contrast media for computerized tomography of liver and spleen. Our accumulated experience with specific carcinogens has also enabled us to initiate studies on the prevention or reversal of chemically induced tumors. This project is of great significance to biomedical research in general, and continues to serve well the objectives of the Program.

Publications:

1. Sieber, S. M., Correa, P., Dalgard, D. W., McIntire, K. R., and Adamson, R. H.: Carcinogenicity and hepatotoxicity of cycasin and its aglycone, methylazoxymethanol acetate, in nonhuman primates. JNCI 65: 177-189, 1980.
2. Vermess, M., Doppman, J. L., Sugarbaker, P., Fisher, R. I., Chatterji, D. C., Luetzeler, J., Grimes, G., Girton, M., and Adamson, R. H.: Clinical trials with a new intravenous liposoluble contrast material for computed tomography of the liver and spleen. Radiology 137: 127-222, 1980.
3. Adamson, R. H., and Sieber, S. M.: Chemically-induced leukemia in humans. Environ. Health Perspect. 1981 (in press).
4. Adamson, R. H., and Sieber, S. M.: Chemical Carcinogenesis Studies in Nonhuman Primates. In: Proceedings of a Symposium on Organ and Species Specificity in Chemical Carcinogenesis. 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06108-12 LCHP																		
PERIOD COVERED October 1, 1980 to September 30, 1981																				
TITLE OF PROJECT (80 characters or less) Studies on the Mechanism of Action and Mechanism of Resistance of Antitumor Agents																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																				
<table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Richard L. Cysyk</td> <td style="width: 33%;">Head, Drug Metabolism Section</td> <td style="width: 33%;">LCHP NCI</td> </tr> <tr> <td>Other: Paul E. Gormley</td> <td>Medical Officer</td> <td>LCHP NCI</td> </tr> <tr> <td>D. Dale Shoemaker</td> <td>Sr. Staff Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td>John M. Strong</td> <td>Sr. Staff Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td>Michael E. McManus</td> <td>Visiting Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td>Louis Malspeis</td> <td>Professor</td> <td>Ohio State University</td> </tr> </table>			PI: Richard L. Cysyk	Head, Drug Metabolism Section	LCHP NCI	Other: Paul E. Gormley	Medical Officer	LCHP NCI	D. Dale Shoemaker	Sr. Staff Fellow	LCHP NCI	John M. Strong	Sr. Staff Fellow	LCHP NCI	Michael E. McManus	Visiting Fellow	LCHP NCI	Louis Malspeis	Professor	Ohio State University
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SUMMARY OF WORK (200 words or less - underline keywords) In studies relating to the toxic effect of <u>misonidazole (NSC-261037)</u> in hypoxic cells, it was found that <u>covalent attachment of C¹⁴-misonidazole metabolites to proteins occurred after activation by rat liver microsomes or purified NADPH-cytochrome C reductase.</u> Studies on the mechanism of action of <u>m-AMSA (NSC-249992)</u> were extended. The metabolites of m-AMSA (m-AQI and m-AQDI) were found to be approximately 100-fold more cytotoxic than m-AMSA. Analogues of m-AMSA with substitutions in the anilino nitrogen were non-toxic, supporting the role of <u>N-hydroxylation</u> in the bioactivation of m-AMSA. Studies comparing the relative <u>DNA strand breakage</u> capability of m-AMSA, its analogues, and its metabolites are continuing. Also, a study of the microsomal metabolism of <u>o-AMSA</u> (an inactive analogue of m-AMSA) was initiated to gain information on the importance of bioactivation to the cytotoxic effects of m-AMSA.																				

Project DescriptionObjectives:

A knowledge of the mechanism of action of anticancer agents and why cells become resistant to them will aid in the design of new chemotherapeutic drugs and will aid in the treatment of neoplastic diseases. Specifically:

To determine the mechanism of action of various anticancer agents.

To determine the mechanism of resistance to various anticancer agents.

To rationally design new anticancer agents.

Methods Employed:

Usual biologic, pharmacologic, and biochemical techniques. Among these are tumor transplantation, harvesting of cells, tissue culture techniques, separation and analyses of whole cell DNA, RNA, and protein synthesis. Various enzymes are isolated and purified, and the effect of various antitumor agents as inhibitors of these enzymes are studied in addition to effects on DNA, RNA and various proteins. Molecular models are also used in drug design.

Major Findings:Covalent Binding of Misonidazole (NSC-261037) after Activation by Rat Liver Microsomes and Purified NADPH-Cytochrome C Reductase.

Misonidazole, a radiation sensitizer of hypoxic cells, also exhibits preferential toxicity towards hypoxic mammalian cells. Certain investigators have speculated on a link between the cytotoxicity of misonidazole to hypoxic cells and the occurrence of neurotoxicity in patients following high dose misonidazole therapy. While the exact mechanism of misonidazole toxicity is unknown, it has been suggested that metabolic reduction of the nitro group to a reactive species is required, as is the case with other aromatic nitro compounds. Studies using [^{14} C] labelled misonidazole were undertaken in rat liver microsomes to determine whether misonidazole is enzymatically converted to metabolites sufficiently reactive to bind to tissue macromolecules. The major findings were that [^{14} C]-misonidazole was metabolized by rat liver microsomes and purified NADPH-cytochrome C reductase anaerobically to a reactive intermediate that covalently binds to tissue macromolecules. Air strongly inhibited the binding whereas carbon monoxide had no effect, indicating that misonidazole is activated via reduction and not by cytochrome P450 dependent oxidation. Both systems showed an absolute requirement for NADPH and were stimulated by flavine (FAD) and paraquat. The apparent K_m for misonidazole binding to microsomal protein was 0.74mM and the apparent V_{max} was 0.64nmoles ^{14} C bound/mg/min. At a single substrate concentration nitrofurantoin, nitrofurazone and desmethyl-misonidazole, a known metabolite of misonidazole, inhibited the covalent binding of misonidazole to protein by 47, 26, and 38%, respectively. Glutathione reduced the binding of misonidazole to microsomal protein below the level observed for boiled microsomes while ascorbic acid had no effect. Compared to nitrofurantoin

and paraquat, misonidazole was a poor stimulator of superoxide production as measured by adrenochrome formation.

Studies on the Mechanism of Action of m-AMSA (NSC-249992).

Studies on the metabolism of m-AMSA by rat liver microsomes demonstrated that two products are formed: N₁-methanesulfonyl-N₄-(9-acridinyl)-3-methoxy-2,5-cyclohexadiene-1,4-dimine (m-AQDI) and 3-methoxy-4-(9-acridinylamino)-2,5-cyclohexadien-1-one (m-AQI). An *in vitro* cell colony assay in soft agar utilizing L1210 cells was established to determine the relative cytotoxicities of m-AMSA and its metabolites, m-AQDI and m-AQI. When L1210 cells were exposed to varying concentrations of m-AMSA for varying lengths of time it was found that cell-kill was dependent on concentration and length of exposure up to 4 hours. Preliminary experiments with m-AQDI and m-AQI indicate that these compounds are approximately 100-fold more toxic than m-AMSA, and, unlike m-AMSA, toxicity was not time-dependent. The principal *in vivo* biliary metabolite of m-AMSA, m-AMSA-5-glutathione, was not toxic. Derivatives of m-AMSA were synthesized in which the nitrogen of the anilino ring was substituted to form a tertiary amine. These derivatives were non-toxic, supporting our hypothesis that N-hydroxylation is the initial reaction in the metabolism of m-AMSA. These results indicate that m-AMSA is bioactivated with time *in vivo* to a highly reactive species capable of reacting with critical macromolecules causing cell death, or with molecules such as glutathione resulting in detoxification.

O-AMSA, an analogue of m-AMSA, is devoid of antitumor activity and has reduced *in vitro* cytotoxic activity. Previous biochemical and biophysical studies have failed to distinguish properties of m-AMSA and o-AMSA that would account for their difference in activity. However, the oxidation products of o-AMSA (o-AQDI and o-AQI), are as cytotoxic as m-AQDI and m-AQI. If o-AMSA is inactive because it is not bioactivated, then the activity of m-AMSA would be related to its intracellular bioactivation. O-AMSA was found to be metabolized in a system comprised of microsomes, oxygen, and a NADPH generating system. The need for cytosol was less critical than in m-AMSA metabolism. At equimolar concentrations, o-AMSA was metabolized at a significantly greater rate than m-AMSA. o-AMSA was found to competitively inhibit the metabolism of m-AMSA, while m-AMSA appeared to non-competitively inhibit the metabolism of o-AMSA. Thus, o-AMSA and m-AMSA appear to be metabolized by different enzymes. Further evidence for this was obtained in kinetic experiments. The apparent K_m for o-AMSA metabolism was $1 \times 10^{-5} M$, while the V_{max} was 40 nmoles/2mg/15min, approximately 6 fold greater than the V_{max} for m-AMSA. Furthermore, the main biliary metabolite of o-AMSA in rats demonstrated an R_f differing from that of m-AMSA as determined by TLC. These preliminary studies indicate that o-AMSA may be metabolized by a different microsomal pathway than m-AMSA and that this could account for the difference in antitumor activity. We are currently attempting to identify the microsomal metabolites of o-AMSA.

Work on the interaction of DNA with m-AMSA has progressed slowly since the last annual report. The attempt to detect m-AMSA covalently attached to the DNA of L1210 cells was unsuccessful. The sensitivity of the assay would have revealed

one AMSA molecule bound for each $1.0-1.5 \times 10^4$ DNA base pairs, but no binding could be shown. Further studies on the interaction of activated AMSA intermediates confirmed that m-AMSA, m-ADQI, and m-AQI cause DNA strand breaks. Similar studies on the o-AMSA series revealed that, while o-AMSA caused no DNA breaks, the activated intermediate o-AQI was capable of inducing DNA breaks in L1210. The generation of DNA strand breaks by m-AMSA appears to require an intact cell. Continuing efforts are being made to find an *in vitro* non-cellular system in which m-AMSA can be shown to cause DNA breaks. These efforts, mostly centered on isolated hepatic microsomes, have encountered difficulties because of the spontaneous DNA breaks induced by the microsomes themselves. Investigations on methods to reduce this background DNA breakage are in progress.

Publications:

1. Karle, J. M., Csyk, R. L., and Karle, I. L.: Structural comparison of m-AMSA, a new clinically active antitumor agent, with less active related compounds. Acta Crystallogr. (B)36: 3012-3016, 1980.
2. Sinha, B. K. and Csyk, R. L.: Interactions of N^2 -substituted spinlabeled analogs of actinomycin-D with nucleic acids and erythrocyte ghost membranes. Chem. Biol. Interact., 1981 (in press).
3. Kensler, T. W., Mutter, G., Hankerson, J. G., Reck, L. J., Harley, C., Han, N., Ardalan, B., Csyk, R. L., Johnson, R. K., Jayaram, H. N., and Cooney, D. A.: Studies on the mechanism of resistance of variants of the Lewis lung carcinoma to N-(phosphonacetyl)-L-aspartic acid. Cancer Res. 41: 894-904, 1981.
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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06134-06 LCHP																				
PERIOD COVERED October 1, 1980 to September 30, 1981																						
TITLE OF PROJECT (80 characters or less) The Role of the Lymphatic System in the Absorption and Distribution of Antitumor Agents																						
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SUMMARY OF WORK (200 words or less - underline keywords) The role of the <u>lymphatic system</u> in the absorption and distribution of free and liposome-entrapped antitumor agents (<u>adriamycin</u> , <u>ara-C</u> and <u>melphalan</u>) administered to rats by ip, iv or sc routes is under investigation. Liposomal encapsulation markedly altered many pharmacological properties, such as tissue distribution, metabolism and excretion, of the drugs tested; entrapment in liposomes also produced a significant increase in their lymphatic absorption and lymph node uptake. The antitumor effect of melphalan against lymph node metastasis of <u>mammary adenocarcinoma 13762</u> was enhanced by liposomal encapsulation. Studies in progress are aimed at optimizing <u>liposome characteristics</u> (e.g., size, charge and lipid composition) for maximal lymph node uptake and retention and for maximal chemotherapeutic activity.																						

Project Description:Objectives:

The objective of this project is to obtain information on the role of the lymphatic system in the absorption and distribution of clinically useful antitumor agents following their administration by subcutaneous, intravenous or intraperitoneal routes. The development of methods for producing a preferential and selective uptake of antitumor agents by lymphatic vessels and lymph nodes is a major goal in this work. Efforts are currently focused on evaluating liposomal encapsulation of drugs as a means of attaining this goal. These studies are designed to determine the relative rate and extent to which free and liposome-entrapped antitumor agents are removed from the peritoneal cavity and interstitial space by lymphatic versus blood routes. In addition, the effect of liposomal entrapment of antitumor agents on other pharmacologic properties, such as tissue disposition, toxicity and antitumor activity, is being assessed. Reproducible model systems in rodents for tumor metastasis via lymphatic channels to regional lymph nodes are being developed and evaluated so that it will be possible to assess the therapeutic advantage of treating such tumors with antitumor agents targeted by liposomal encapsulation to regional lymph nodes.

Major Findings:

Liposomes administered by the ip route are relatively extensively absorbed by lymphatics. Liposomes were prepared using ^3H -dipalmitoyl-phosphatidylcholine (DPPC) or ^{14}C -cholesterol as lipid markers; in some studies fluorescent or radiolabeled substances were included to serve as markers for the aqueous space. Following ip treatment with "empty" ^3H -DPPC/cholesterol/stearylamine (3:3:1 molar ratio) liposomes, 30% or more of the administered tritium was recovered in 24-hour thoracic duct lymph. Lymph radioactivity was 10-40 times higher than plasma at intervals up to 3 hours after dosing. Since these preliminary studies indicated that liposomes administered to rats by ip injection were relatively extensively absorbed by lymphatics, it seemed possible that the concentration of various antitumor agents in lymphatic channels might be enhanced by entrapping the agents in liposomes.

Therefore, the lymphatic absorption and tissue distribution of free ^{14}C -adriamycin and ^{14}C -adriamycin entrapped in ^3H -liposomes, was examined at intervals after ip injection into rats. Following treatment with free ^{14}C -adriamycin only 1% of the dose was recovered in 24-hr lymph, and liposomal encapsulation produced a six-fold increase in this value. Liposomal encapsulation of adriamycin altered its tissue disposition, chiefly by increasing the concentration of drug equivalents in diaphragm, liver, and spleen. Although free adriamycin was accumulated by lymph nodes to some extent, lymph node uptake was markedly enhanced by liposomal encapsulation and was present only in those nodes through which lymph draining the peritoneal cavity passes. This finding, together with the observation that diaphragm and thoracic duct lymph contained relatively high levels of liposomal lipid and adriamycin equivalents, indicates that liposomes are selectively absorbed from the peritoneal cavity by lymphatics and are retained by certain lymph nodes. The results of this study suggest that ip administration of liposome-encapsulated drugs may provide a means of selectively

concentrating antitumor agents in lymphatic channels and lymph nodes.

The tissue distribution and lymph node uptake of liposome-encapsulated ^{14}C -adriamycin administered to rats by the iv route was compared with results obtained after ip dosing. Regardless of the route of administration, the uptake of adriamycin by liver and spleen was increased 2-10 fold by liposomal encapsulation at 4 and 24 hours post treatment; in contrast, liposomal encapsulation decreased adriamycin uptake by heart, lung, skeletal muscle, kidney and gut 2-6 fold after either ip or iv dosing. Drug levels in these tissues were up to 3 times higher after iv than after ip dosing at 4 hours after treatment, although at 24 hours tissue levels were ~ 2 times higher in ip treated rats than in rats dosed by the iv route. Twenty-four hour biliary excretion of radioactivity was 2-3 fold lower in rats treated ip and iv with liposome-encapsulated adriamycin than with the free drug. This may in part explain the increased persistence of radiolabel in plasma and certain other tissues noted in rats treated by both routes with encapsulated, versus free, adriamycin. The most striking difference between ip and iv routes with regard to tissue distribution was found in diaphragm and lymph nodes. Following an ip dose of free adriamycin, drug levels in diaphragm and thoracic lymph nodes were higher by factors of 10 and 75, respectively, than in corresponding plasma at 4 and 24 hours after dosing, and this accumulation was increased 4-fold by liposomal encapsulation. In contrast, free adriamycin administered by the iv route did not accumulate in either diaphragm or lymph nodes, and liposomal encapsulation reduced the uptake of adriamycin in these tissues by 25-50% at 4 and 24 hours after dosing. These results raise the possibility of attaining increased drug concentrations in diaphragmatic lymph channels and in lymph nodes by utilizing the ip route for drug administration; moreover, they indicate that liposomal encapsulation increases drug uptake into lymphatic channels and lymph nodes following ip, but not iv, dosing.

Studies on the lymphatic absorption and tissue distribution of free and liposome-entrapped ara-C have been completed. Unlike free adriamycin, free ara-C does not have an affinity for elements of the reticuloendothelial system including lymph nodes. Free [^{14}C]ara-C was completely absorbed from the peritoneal cavity of thoracic duct cannulated rats by 6 hr after ip dosing. ^{14}C -levels in most tissues were higher at 4 hr than at 12 hr after dosing and were generally undetectable at 24 hr. By 6 hr post treatment only 2% of the dose was recovered in lymph, whereas 90% had been excreted in urine. Liposome entrapment of ara-C reduced the rates at which the drug was absorbed from the peritoneal cavity and excreted in urine while enhancing lymphatic uptake of the drug by more than 10-fold. Radioactivity in plasma and most tissues achieved higher concentrations and persisted for longer periods in rats given liposome entrapped ara-C than in rats receiving the free drug. Most striking was the localization of ^{14}C -activity in renal and thoracic lymph nodes of rats given liposome entrapped ara-C, with 300-1000 fold higher levels present at 4, 12 and 24 hr post treatment than in corresponding lymph nodes of rats receiving the free drug. The metabolic conversion of ara-C to uracil- β -D-arabinofuranoside was reduced by ~ 3 fold following liposome entrapment of the drug. As noted for liposome-entrapped adriamycin, the enhanced lymphatic uptake and the localization and persistence of ara-C in lymph nodes resulting from liposome entrapment of the drug may be of benefit in treating tumors that metastasize via lymphatic pathways.

The relationship of liposome size to extent of lymphatic absorption and lymph node retention is under evaluation in rats. Large unfiltered liposomes and liposomes formed by extrusion through 0.6 μ and 0.2 μ filters are administered by ip injection to rats, and their clearance from the peritoneal cavity and uptake by lymphatics measured at intervals thereafter. Thus far it appears that the peritoneal absorption of unfiltered liposomes is impeded by their large size; those liposomes that are cleared from the peritoneal cavity are taken up by renal and thoracic nodes where they are retained for prolonged periods. Smaller liposomes, those extruded through 0.6 μ filters, leave the peritoneal cavity more rapidly than unfiltered liposomes, but their retention in lymph nodes is not as prolonged. These studies are continuing in order to define the optimal liposome size for rapid peritoneal clearance but prolonged lymph node retention.

In order to assess the therapeutic effects of liposome-encapsulated antitumor agents against lymphogenous metastases, it has been necessary to establish a model for lymph node metastasis which is reproducible and quantitative. Two rat tumors, Walker 256 carcinoma and 13762 mammary adenocarcinoma, have been developed for this purpose. Tumor cell suspensions are inoculated subcutaneously in the hind leg of rats, and at various intervals after inoculation the tumor-bearing leg is amputated. For both tumors, metastasis takes place by lymphatics to regional lymph nodes and by hematogenous spread to lungs. For the mammary adenocarcinoma 13762, the time interval between inoculation and amputation has been found to be a critical determinant in the extent of lymph node involvement with tumor, as determined by serial measurement of lymph node weight or volume, and in the survival time of the animal. Amputation prior to 5 days after tumor inoculation reduces the incidence of blood-born lung metastases but also renders the development of lymph node metastasis less reproducible. Amputation on day 9 or 10 after tumor inoculation results in regional lymph node metastases in 100% of the animals at risk, and the weight of the lymph node has been found to reflect the degree of tumor involvement; however, the animals ultimately die of lung metastases.

The effect of liposomal entrapment of melphalan (MPL) on its tissue distribution and activity against lymph node metastasis of the 13762 adenocarcinoma was evaluated in rats. Neutral small liposomes containing MPL and the fluorescent marker carboxyfluorescein (CF) were prepared by sonication using phosphatidylcholine (PC) and cholesterol (molar ratio, 2:1), along with tracer amounts of ^{14}C -MPL and ^3H -PC. Unlike CF, MPL appears to associate with the lipid phase of liposomes; entrapped MPL was not hydrolyzed, and was gradually released from liposomes during incubation at 37°C. Free ^{14}C -MPL injected sc into the thigh of rats was rapidly cleared from the injection site, with only 0.6% of the ^{14}C activity remaining after 2 hours. The concentration of MPL in the ipsilateral inguinal lymph nodes showed a transient increase over that in contralateral nodes at 0.5 hour, although by 1 hour MPL concentrations were similar in ipsi- and contralateral nodes. After sc injection of ^{14}C -MPL/ ^3H -PC-liposomes, about 4% of the ^{14}C activity was present at the injection site at 2 hour post treatment. The concentration of PC equivalents in ipsilateral lymph nodes was at least 50 times higher than in plasma, lung or contralateral lymph nodes at all time intervals examined. Similarly, liposomal entrapment of MPL enhanced its uptake by ipsilateral lymph nodes; this increase in lymph node MPL concentration

was sustained for at least 24 hours after dosing, at which time ipsilateral nodes contained 20-fold and 10-fold higher MPL levels than were present in plasma and contralateral nodes, respectively. Rats bearing the 13762 adenocarcinoma were given a single sc injection of free or liposome-entrapped MPL 3 days after surgical resection of the primary tumor. Liposome-entrapped MPL produced a greater reduction in the weight of lymph node metastases than did free MPL. Thus, when MPL was entrapped in liposomes, a dose of 0.125 mg/kg reduced the weight of lymph node metastases to about 50% of controls. A dose of 0.5-1.0 mg/kg was required to exert an equivalent effect on lymph node tumor growth when free MPL was administered. These results suggest that the interstitial injection of liposomes containing MPL may be useful for the postoperative treatment of lymph node metastases.

The ability of several proteolytic enzymes, administered concomitantly with MPL-liposomes, to hasten clearance of liposomes from the sc injection site and enhance regional lymph node uptake is being evaluated. Hyaluronidase, collagenase and chondroitinase, singly and in combinations, have thus far been tested. Hyaluronidase was most effective in increasing the clearance rate of liposomes from the sc injection site and produced a 3-fold increase in the uptake of liposomes by the regional lymph node at 2 hours after dosing; the concentration of hyaluronidase used (100-200 IU/ml) did not produce histologic evidence of tissue damage at the injection site, nor did it appear to alter the integrity of the liposomes themselves. Thus the use of proteolytic enzymes to break down the interstitial matrix and/or basement membrane may be a feasible means of enhancing lymph node uptake of liposomes from the interstitial space.

Significance to Biomedical Research and the Program of the Institute:

Liposomal encapsulation is a novel drug delivery system; this project represents an attempt to improve the selectivity of antitumor agents by targeting them, through liposomal encapsulation, to specific sites. Tumor metastasis is a major clinical problem in treating cancer. Lymphatic channels are frequently the route by which tumors metastasize, with micrometastases lodging in regional and even distant lymph nodes. It would therefore be of potential therapeutic advantage in the treatment of early metastases to selectively concentrate antitumor agents in lymph channels and lymph nodes. The results of our studies suggest that liposomal encapsulation is a feasible method for targeting antitumor agents to these sites.

Proposed Course:

To continue to pursue the goals listed under "Objectives" above. Particular emphasis will be given to optimizing liposomal characteristics such as size and lipid composition for maximal uptake by lymphatics and lymph nodes. Efforts will also continue to be directed toward developing new experimental models for lymphatic metastasis, improving existing model systems, and utilizing these models for evaluating the therapeutic effects of liposome-entrapped antitumor agents.

Publications:

1. Parker, R. J., Hartman, K. D., and Sieber, S. M.: Lymphatic absorption and tissue disposition of liposome-entrapped [^{14} C]Adriamycin following intraperitoneal administration to rats. Cancer Res. 41: 1311-1317, 1981.
2. Parker, R. J., Sieber, S. M. and Weinstein, J. N.: The effect of liposome encapsulation of a fluorescent dye on its uptake by the lymphatics of the rat. Pharmacology, 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06136-05 LCHP																										
PERIOD COVERED October 1, 1980 to September 30, 1981																												
TITLE OF PROJECT (80 characters or less) Chemically Induced Cell Differentiation in Neoplasia																												
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SUMMARY OF WORK (200 words or less - underline keywords) This study was initiated to understand the mechanism of <u>chemically induced cell differentiation in neoplasia</u> . The research is directed at present to determine: (1) the effect of <u>dimethylsulfoxide (DMSO)</u> , <u>bisacetamides</u> , <u>hormones</u> , and other selective compounds (<u>including antitumor agents</u>) on the expression of <u>oncofetal markers in hepatoma and leukemia cell lines</u> from animals and humans; (2) the expression of <u>oncofetal markers in premalignant hepatocyte populations</u> ; (3) the <u>reversibility</u> of the neoplastic state when these cells and cell lines are exposed to the above mentioned agents.																												

Project Description:Objectives:

The objective of this project is to examine the feasibility of using chemically induced cell differentiation as a therapeutic approach to the treatment of neoplastic diseases either alone or in combination with established chemotherapeutic agents. This approach is aimed at finding chemicals with the capacity to "redifferentiate" or normalize transformed malignant cells. Information already available indicates that this approach is feasible. For example, the addition of DMSO to cultures of murine virally-transformed erythroleukemia cells results in erythroid differentiation characterized by the appearance of globin mRNA, synthesis of hemoglobin, cessation of cell division, appearance of erythrocyte membrane antigens, and the morphological changes characteristic of erythroid differentiation in normal mouse hemopoietic tissue. Currently we are using rat hepatoma cell lines, murine erythroleukemia cell lines and several human leukemic cell lines in our studies.

Methods Employed:

Methods used in these studies include: tissue culture techniques, radioimmunoassays, differential centrifugation, radioisotope measurements using tritium, carbon-14, and iodine-125; phase contrast microscopy, photomicroscopy; enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations; two dimensional electrophoresis; and chemical synthesis.

Major Findings:

1. Two hepatoma cell lines McA-RH 7777 (7777) and McA-RH 8994 (8994) were treated with hexamethylene bisacetamide (HMBA) to assess its effects on the production of alpha fetoprotein (AFP), albumin and transferrin. Radioimmunoassays were used to determine the levels of both secreted and intracellular concentrations of AFP, albumin and transferrin. 7777 normally produces large amounts of AFP but the production of albumin is very low in this cell line, and it therefore resembles the fetal liver with respect to the secretion of these two proteins. In contrast 8994 produces large amounts of albumin and very small quantities of AFP thus resembling hepatic functions characteristic of a more differentiated state. After a period of 28 to 36 hours of HMBA treatment a 3 fold increase in AFP secretion by 7777 and a dose related increase in AFP, albumin and transferrin secretion by 8994 were observed. However, in 7777 HMBA treatment decreased the secretion of albumin and transferrin to 40% and 75% respectively of control values. The intracellular concentration of AFP in 7777 and all three proteins in 8994 was increased by treatment with HMBA indicating that this agent is able to stimulate the synthesis of these proteins. The intracellular concentration of transferrin produced by 7777 was decreased by treatment with HMBA indicating an inhibition of transferrin synthesis. However, while secretion of albumin was decreased by HMBA treatment of 7777 intracellular albumin increased in this cell line indicating a limited secretory capacity for this protein and/or a stimulation of albumin synthesis which is masked by a limited secretory process.

HMBA stimulates the production of the oncofetal protein AFP, a result which appears to be independent of the stage of differentiation of the cell. However, its effect on albumin and transferrin are opposite in the two cell lines, suggesting that the production of these two proteins are controlled by factors or conditions which are dependent upon the stage of differentiation of the cell.

2. Established human lymphoid cell lines are useful models for the study of lymphoid differentiation. We are currently studying several unique undifferentiated lymphoma cell lines which are EBV negative and contain the 8q⁺, 14q⁺ translocation. The lines have been analyzed for the presence of surface and cytoplasmic Ig in addition to surface receptors for EBV, complement (C), and the Fc portion of IgG. Furthermore 2D gel electrophoresis (O'Farrell) was employed to compare patterns of cellular proteins among the cell lines. The lines differ considerably in expression of cell surface markers and their ability to be induced for the same with pharmacologic agents. Two lines derived from patient JD are of particular interest; one line derived from the original tumor JD-A and another (JD-PB) was obtained at relapse. JD-PB possesses increased numbers of C receptors compared to JD-A (25% vs 0-10%) and is much more readily infected with EBV suggesting increased numbers of EBV receptors. In addition 2D gel electrophoresis of total cellular proteins demonstrates several differences between the lines. High levels of C receptors (50-90% of cells) were readily induced in the JD-PB line by such agents as hexamethylenebisacetamide (5×10^{-3} M), DMSO (2×10^{-1} M), ascorbate (3×10^{-4} M), dexamethasone (10^{-5} M), Theophylline (6×10^{-3} M), and butyric acid (1×10^{-3} M). Similarly, the JD-A line was induced, but to much lesser extent, with hexamethylenebisacetamide and butyric acid. Theophylline has no effect upon JD-A C receptor induction. These data suggest that the JD-A and JD-PB lines are clones that differ both with respect to stages of differentiation and the capacity to be induced to differentiate by pharmacological agents.
3. The effects of inducers of cellular differentiation, such as DMSO and butyric acid (BA) on membrane microviscosity has been determined by ESR spectroscopy. Friend's erythroleukemia cells were grown batchwise in stock media, in stock media with 2% DMSO and in stock media with 2 mM BA. After 6 days the cells were harvested by centrifugation (2000 x G for 10 min., Beckman Model TJ-6 centrifuge), washed three times in 50 mM Tris buffer (pH 7.5) and resuspended in 50 mM Tris buffer (pH 7.5) at an approximate ratio of 0.5 ml of packed cells to 100 μ l of buffer. Membrane viscosity studies were conducted using ESR measurements of the spin probe 5-doxylstearic acid (1-oxy-2,2-dimethyloxazolidine derivative of 5-ketostearic acid). Studies were performed on a Varian E9, X-band spectrometer equipped with a variable temperature control. The 5-doxylstearic acid (5NS) was incorporated into the cell membranes by adding the resuspended cells to a tube in which 10 μ l of the probe had been evaporated to dryness. The cell suspension was swirled over the surface of the tube for 30 seconds. The tube was then placed in ice from 30 minutes to 1 hour to allow maximum incorporation of the probe and to prevent rapid reduction of the signal at higher temperatures. Spectra were read at 10, 20, 25, 30, 35 and 40°C.

Cells that had been differentiated by both DMSO and BA showed marked decreases in membrane microviscosity as shown by ESR spin label studies. Preliminary data indicate that microviscosity changes are not a steady transition from control levels to differentiated levels. Rather a decrease in microviscosity appears to occur during the first 24 hours of exposure to the inducing agent after which progressive increases in microviscosity are seen.

4. We have currently been employing the technique of 2 dimensional electrophoresis to analyze changes in gene expression during differentiation of both virally and chemically transformed cells (erythroleukemia, hepatomas, and lymphomas). This is an extremely sensitive technique capable of detecting over a 1000 distinct proteins from cellular extracts. We have been using 2 clones (namely C19TK and 5-19) of Friends erythroleukemia cells to study the terminal differentiation of these cells to hemoglobin producing cells. Both lines can be induced to differentiate to hemoglobin producing cells following treatment with a wide variety of chemical inducing agents. Three such agents are dimethylsulfoxide (DMSO) hexamethylene bisacetamide (HMBA) and butyric acid (BA). In addition erythrodifferentiation induced by these chemicals can be blocked by the corticosteroids, hydrocortisone (HC) and dexamethasone (Dex). Addition of HC is time dependent and HC can be added as late as 48 hrs after DMSO treatment (time at which cells are committed to differentiate) and erythrodifferentiation is still markedly inhibited. In addition to inhibition by HC and Dex the β adrenergic blocker, dl-propranolol is also an excellent inhibitor of both DMSO and HMBA induced erythrodifferentiation in 5-18 cells but not in C19TK cells. Propranolol shows a similar time course of inhibition in 5-18 cells as does HC and modulation of the β adrenergic receptor is apparently not involved in the inhibition of erythrodifferentiation.

Using 2-dimensional electrophoresis we are now analyzing the relative rates of synthesis of proteins from total cellular extracts (both membrane and cytosolic proteins) from untreated cells and cells treated with DMSO, HMBA, DMSO/HC, DMSO/Prop, HMBA/HC, and HMBA/Prop. Following treatment, 5-18 and C19TK cells are pulse labelled with either [^{35} S] methionine (200 μ Ci/ml) or [^{14} C] labelled amino acid mixtures (50 μ Ci/ml) for 30 mins and total cellular extracts are made. Labelled proteins (300,000 dpm) are subjected to isoelectric focusing in the first dimension and are then separated on the basis of molecular weight in the second dimension. Following either autoradiography or fluorography of the dried gels qualitative and quantitative changes in protein patterns are analyzed using GELLAB, a computer system specially written for the analysis of 2D gel electrophoresis images on a DECSYSTEM 2020 computer. In order to quantitate changes in protein synthesis we have prepared calibration strips of polyacrylamide gel with 10-12 segments, each segment containing a known amount of radioactivity (0.1-200 dpm/mm²). Gels are routinely exposed to MR-1 X-ray film for both 3 days (detects most abundant proteins) and 7 days (minor proteins) and comparisons are made between the gels. Preliminary results have indicated both qualitative and quantitative changes following induction of erythrodifferentiation by DMSO and HMBA in both 5-18 and C19TK cells.

Proposed Course:

Continue the course outlines under Objectives.

Publications:

1. Schut, H. A. J., Hughes, E. H., and Thorgeirsson, S. S.: Differential effects of dimethyl sulfoxide and sodium butyrate on α -fetoprotein, albumin and transferrin production by rat hepatomas in culture. In Vitro, 1981 in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06137-05 LCHP																						
PERIOD COVERED October 1, 1980 to September 30, 1981																								
TITLE OF PROJECT (80 characters or less) Mechanism of Chemically Induced Hepatomas and Ovarian Tumors																								
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SUMMARY OF WORK (200 words or less - underline keywords) The long range purpose of this project is to study the mechanism of <u>chemically induced hepatomas and ovarian tumors</u> . We are particularly interested in the role of <u>environmental contaminants</u> such as the <u>polycyclic hydrocarbons, nitro-so-compounds, and N-acetylarylamines</u> , and of <u>antitumor agents</u> in initiating these tumors, as well as the role of <u>genetic predisposition</u> in tumor development. These tumors, at various time points during their development, will be used as models for evaluating <u>chemotherapeutic agents</u> and other drugs which might be of <u>therapeutic value</u> . Topics of present interest are: (1) the role of <u>metabolic activation</u> , as well as the <u>genetic control</u> of activating enzymes, in the chemical initiation of hepatomas and ovarian tumors; (2) the <u>time course</u> of hepatoma and ovarian tumor formation with particular emphasis on the changing enzyme patterns as the cells become increasingly dedifferentiated; (3) isolation and characterization of <u>pre malignant cell populations</u> in the liver.																								

Project Description:Objectives:

The main objectives of this project are: (1) to study both in vivo and in vitro the relationship between the activity of the enzymes which metabolically activate compounds such as polycyclic hydrocarbons, N-arylamines and nitroso-compounds, and the carcinogenicity of these compounds. The main emphasis will be focused on the liver and the ovary; (2) to study the time course of hepatoma formation in which the main emphasis will be on the isolation and characterization of preneoplastic cells with respect to changing enzyme patterns and malignant potential.

Methods Employed:

The principal methods employed are: (1) recording spectrophotometry of turbid solutions, (2) enzyme assays involving radiometric, spectrophotometric, and spectrophotofluorometric determinations of product formation or substrate disappearance, (3) radioisotopic measurements using tritium and carbon-14, (4) differential centrifugation, (5) polyacrylamide gel electrophoresis, (6) column and thin-layer chromatography, (7) high pressure liquid chromatography, and (8) bacterial culture technique.

Major Findings:

1. Cyclophosphamide, azathioprine and 6-mercaptopurine were studied for oocyte and follicle toxicity in a murine ovarian toxicity assay system. Seven days after a single treatment with cyclophosphamide (100 mg/kg, ip), 63% of the primordial follicles and 0% of the growing or large follicles and their oocytes were destroyed in four week old C57BL/6N mice. Treatment with azathioprine (100 mg/kg/day, ip x 9 days) or 6-mercaptopurine (100 mg/kg/day, ip x 9 days) suppressed weight gain and increased mortality but had no effect on the number of oocytes or follicles in these mice. These data are consistent with previous observations on the effects of cyclophosphamide, azathioprine and 6-mercaptopurine on the human ovary. This suggests that the murine ovarian toxicity assay system may be useful in evaluating xenobiotics, including drugs, for human ovarian toxicity.

2. A high pressure liquid chromatography method utilizing desferal mesylate as a solvent additive was developed for the simultaneous separation of 2-acetylaminofluorene, N-hydroxy-2-acetylaminofluorene, 9-hydroxy-2-acetylaminofluorene, 7-hydroxy-2-acetylaminofluorene, 5-hydroxy-2-acetylaminofluorene, 2-aminofluorene, and 2-acetylaminofluorene-9-one. The method was used to quantitate these metabolites formed when 2-acetylaminofluorene was incubated with freshly isolated rat liver cells, with rat liver microsomes or with microsomes prepared from isolated hepatocytes. This HPLC method was also used to metabolically characterize preneoplastic hepatocytes that were isolated from rats treated with a combination of diethylnitrosamine, partial hepatectomy and 2-acetylaminofluorene. The isolated hepatocytes were separated into normal and preneoplastic cells on a linear metrizamide gradient. Preliminary data indicate that the regulation of the induction of certain monooxygenase activities by polycyclic hydrocarbons is lost and this may coincide with the expression of α -glutamyl-transpeptidase activity in these preneoplastic cells.

Significance to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing a better understanding of tumor initiation as well as tumor progression. This information will then be used to design chemotherapeutic regimens as well as new chemotherapeutic agents for treating these and other tumors.

Proposed Course of Project:

Continue the course outlined under Objectives.

Publications:

1. Johnson, E. F., Levitt, D. S., Muller-Eberhard, U., and Thorgeirsson, S. S.: Divergent pathways of carcinogen metabolism: metabolism of 2-acetylaminofluorene by multiple forms of cytochrome P-450. Cancer Res. 40: 4456-4459, 1980.
2. Thorgeirsson, S. S., Levitt, D. S., and Smith, C. L.: Induction of N-and C-Hydroxylations of 2-Acetylaminofluorene in Rat Hyperplastic Liver Modules and Hepatomas. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R., and O'Brien, P. J. (Eds): Microsomes, Drug Oxidation, and Chemical Carcinogenesis. New York, Academic Press, 1980, Vol. II, pp. 1181-1185.
3. Mattison, D. R., Menard, R. H. and Thorgeirsson, S. S.: Ovarian Aryl Hydrocarbon Hydroxylase Activity and Oocyte Destruction. In Coon, M. J., Conney, A. H., Estabrook, R. W., Gelboin, H. V., Gillette, J. R., and O'Brien, P. J. (Eds.): Microsomes, Drug Oxidation, and Chemical Carcinogenesis. New York, Academic Press, 1980, Vol. II, pp. 1219-1222.
4. Mattison, D. R., Chang, L., Thorgeirsson, S. S., and Shiromizu, K.: The effects of cyclophosphamide, azathioprine, and 6-mercaptopurine on oocyte and follicle number in C57BL/6N mice. Res. Commun. Chem. Pathol. Pharmacol. 31: 155-161, 1981.
5. Smith, C. L. and Thorgeirsson, S. S.: An improved high pressure liquid chromatographic assay for 2-acetylaminofluorene and eight of its metabolites. Anal. Biochem. 1981 (in press).

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TITLE OF PROJECT (80 characters or less) Mutagenicity and Carcinogenicity of Antitumor Agents and Chemical Carcinogens														
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SUMMARY OF WORK (200 words or less - underline keywords) This project was initiated to evaluate the relationship between <u>chemically induced mutagenesis</u> and <u>carcinogenesis</u> . The primary emphasis is placed on <u>antitumor agents</u> , known <u>environmental contaminants</u> and model <u>chemical carcinogens</u> . A sensitive <u>in vitro microbial test system</u> is used for the detection of chemical mutagens. The research is directed at present to determine: (1) the role, if any, and the mechanism of <u>metabolic activation</u> in the mutagenesis caused by antitumor agents and chemical carcinogens; (2) the <u>predictive value</u> of the <u>in vitro mutagenesis test</u> for <u>in vivo carcinogenesis</u> ; (3) the <u>difference</u> , if any, between the mutagenic effects of antitumor agents and other chemical mutagens in <u>mammalian</u> and <u>microbial</u> cell systems; (4) the effect of structural modification on the mutagenicity of antitumor agents and model chemical carcinogens.														

Project Description:Objectives:

The main objectives of the project are: (1) to examine the role of both sub-cellular fractions and intact rodent hepatocytes in activating procarcinogens to ultimate carcinogens and/or mutagens; (2) to study the relationship between mutagenicity in the in vitro microbial test system (Salmonella test system) and carcinogenicity of antitumor agents and chemical carcinogens; (3) to examine the possible difference in the mutagenic effects of antitumor agents and other chemical mutagens in mammalian and microbial cell systems.

Methods Employed:

The principal methods employed are: (1) bacterial and mammalian culture techniques, (2) differential centrifugation, (3) enzyme assays, (4) recording spectrophotometry, and (5) high pressure liquid chromatography.

Major Findings:

1. The mutagenicity of N-hydroxy-2-acetylaminofluorene and N-hydroxyphenacetin and their respective deacetylated metabolites, N-hydroxy-2-aminofluorene and 2-nitrosofluorene, and N-hydroxyphenetidine and p-nitrosophenetole was determined in nitroreductase deficient Salmonella tester strains TA 98FR and TA 100FR. The mutagenicity of N-hydroxy-2-acetylaminofluorene mediated by either rat liver microsomes or rat liver 105,000g supernatant fractions was no different in either TA 98 (nitroreductase present) or TA 98FR (nitroreductase deficient) strains. Similarly the mutagenicity of N-hydroxyphenacetin mediated by hamster microsomes was not affected by either the presence or absence of nitroreductase activity in TA 100.

N-hydroxy-2-aminofluorene and 2-nitrosofluorene were equipotent direct acting mutagens in both TA 98 and TA 98FR, as were both N-hydroxyphenetidine and p-nitrosophenetole in TA 100 and TA 100FR.

Ascorbate (5 mM) and NADPH (1 mM) had no significant effect on the mutagenicity of either N-hydroxy-2-acetylaminofluorene, N-hydroxy-2-aminofluorene, or 2-nitrosofluorene in TA 98 or TA 98FR whereas ascorbate and NADPH markedly inhibited the mutagenicity of both N-hydroxyphenetidine and p-nitrosophenetole in both TA 100 and TA 100FR. Ascorbate appears to be inhibiting the mutagenicity of N-hydroxyphenetidine and p-nitrosophenetole as a result of the non enzymatic chemical reduction of these compounds to non mutagenic derivatives.

2. Coincubation of isolated intact rat hepatocytes and Salmonella typhimurium tester strains (Salmonella/hepatocyte system) permits determination of both bacterial mutagenicity and DNA damage in the hepatocyte as measured by alkaline elution following treatment with known or suspected chemical carcinogens (BBRC 94, 837-842, 1980). The use of the esterase inhibitor paraoxon in the classical Salmonella system indicates that deacetylation of N-OH-AAF is the primary mutagenic activation pathway for this compound (Mol. Pharmacol. 14, 682-692, 1978). We have determined the effect of paraoxon pretreatment on both bacterial mutagenicity and host cell DNA damage of N-OH-AAF in the Salmonella/hepatocyte

system. Pretreatment with paraoxon over a concentration range from 10^{-4} to 10^{-12} M caused dose related inhibition of the bacterial mutagenicity of N-OH-AAF. However, the effect of paraoxon on N-OH-AAF induced DNA damage was inhibitory at concentrations lower than 10^{-6} M but at higher concentrations of paraoxon the DNA damage was accentuated. These data indicate that paraoxon at low concentration inhibits both bacterial mutagenicity and host cell DNA damage by N-OH-AAF in the Salmonella/hepatocyte system. The host cell DNA damage of N-OH-AAF is increased at higher concentrations of paraoxon whereas the bacterial mutagenicity of N-OH-AAF is still inhibited.

Significance to Biomedical Research and the Program of the Institute:

Our studies are aimed at providing a better understanding of the mechanism of toxicity, mutagenicity and potential carcinogenicity caused by antitumor agents and chemical carcinogens. The information derived from these studies may provide a sounder base for the design of chemotherapeutic regimes, the early identification of individuals at risk to develop cancer, and could provide means to prevent toxic side effects of antitumor agents.

Proposed Course of Project:

Continue the course outlines under Objectives and Major Findings.

Publications:

1. Wirth, P. J., Dybing, E., von Bahr, C., and Thorgeirsson, S. S.: Mechanism of N-hydroxyacetylarylamine mutagenesis in the Salmonella test system: metabolic activation of N-hydroxyphenacetin by liver and kidney fractions from the rat, mouse, hamster, and man. Mol. Pharmacol. 18: 117-127, 1980.
2. Thorgeirsson, S. S., Sakai, S., and Wirth, P. J.: Effect of ascorbic acid on in vivo covalent binding and on in vitro mutagenicity of N-hydroxy-2-acetylaminofluorene in the rat. Mutat. Res. 70: 395-398, 1980.
3. Aune, T., Dybing, E., and Thorgeirsson, S. S.: Developmental pattern of 3-methylcholanthrene inducible mutagenic activation of 2-acetylaminofluorene, 2-aminofluorene and 2,3-diaminoanisole in the rabbit. JNCI 64: 765-772, 1980.
4. Reddy, T. V., Weisburger, E. K., and Thorgeirsson, S. S.: Mutagenic activation of 2-acetylaminofluorene and N-hydroxy-2-acetylaminofluorene in subcellular fractions from X/Gf mice. JNCI 64: 1563-1569, 1980.
5. Staiano, N., Erickson, L. C., and Thorgeirsson, S. S.: Bacterial mutagenesis and host cell DNA damage by chemical carcinogens in the Salmonella/hepatocyte system. Biochem. Biophys. Res. Commun. 94: 837-842, 1980.
6. Reddy, T. V., Benjamin, T., Grantham, P. H., Weisburger, E. K., and Thorgeirsson, S. S.: Mutagenicity of urine from rats after administration of 2,4-Diaminoanisole; the effect of microsomal enzyme inducers. Mutat. Res. 79: 307-317, 1980.

7. Staiano, N., Everson, R. B., Cooney, D. A., Longnecker, D. S., and Thorgeirsson, S. S.: Mutagenicity of D- and L-Azaserine, 6-diazo-5-oxo-L-norleucine and N-(N-methyl-N-nitroso-carbamyl)-L-ornithine in the *Salmonella* test system. Mutat. Res. 79: 387-390, 1980.
8. Timm-Haug, L., Dybing, E., and Thorgeirsson, S. S.: Developmental aspects of 2-acetylaminofluorene metabolism and mutagenic activation in the chick. Xenobiotica 10: 863-873, 1980.
9. Harris, C. C., Mulvihill, J. J., Thorgeirsson, S. S., and Minna, J. D.: Individual differences in cancer susceptibility. Ann. Intern. Med. 96: 809-825, 1980.
10. Wirth, P. J., and Thorgeirsson, S. S.: Mechanism of N-hydroxy-2-acetylaminofluorene mutagenicity in the *Salmonella* test system: role of N-O acyltransferase and sulfotransferase from rat liver. Mol. Pharmacol. 19: 337-345, 1981.
11. Aune, T., Dybing, E., Nelson, S. D., and Thorgeirsson, S. S.: Genetic differences in dimethylnitrosamine mutagenicity in vitro associated with mouse hepatic arylhydrocarbon hydroxylase activity increased by 3-methylcholanthrene. Acta Pharmacol. Toxicol. 48: 118-129, 1981.
12. Staiano, N., Gallelli, J. F., Adamson, R. H., and Thorgeirsson, S. S.: Lack of mutagenic activity in urine from hospital pharmacists admixing antitumor drugs. Lancet 1: 615-616, 1981.
13. Thorgeirsson, S. S., Schut, H. A. J., Staiano, N., Wirth, P. J., and Everson, R. B.: Mutagenicity of N-substituted aryl compounds in microbial systems. JNCI Monogr., 1981 (in press).
14. Dybing, E., Saxholm, H. J. K., Aune, T., Wirth, P. J., and Thorgeirsson, S. S.: Studies on mutagenic and carcinogenic N-substituted aryl compounds--cosmetics and drugs. JNCI Monogr., 1981 (in press).
15. Thorgeirsson, S. S., Wirth, P. J., Staiano, N., and Smith, C. L.: Metabolic Activation of 2-Acetylaminofluorene. In 2nd International Symposium on Reactive Intermediates in Drug Metabolism. Guildford, Surrey, England, 1981 (in press).
16. Boobis, A. R., Brodie, M. J., McManus, M. E., Staiano, N., Thorgeirsson, S. S., and Davies, D. S.: Metabolism and Mutagenic Activation of 2-Acetylaminofluorene by Human Liver and Lung. In 2nd International Symposium on Reactive Intermediates in Drug Metabolism. Guildford, Surrey, England, 1981 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06141-04 LCHP
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less) Kinetics and Pharmacologic Effects of Derivatives of Antitumor Agents		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: D. S. Zaharko Head, Pharmacokin. & Pharmacody. Sec. LCHP NCI Other: L. M. Ramonas Staff Fellow LCHP NCI H. Ringsdorf Prof. Johannes Gutenberg Universitat		
COOPERATING UNITS (if any) Organic Chemistry Department, Johannes Gutenberg Universitat, Mainz, West Germany		
LAB/BRANCH Laboratory of Chemical Pharmacology		
SECTION Pharmacokinetics and Pharmacodynamics Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.25	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Kinetics and pharmacologic effects of <u>activated sulfhydryl derivatives of cyclophosphamide and polymer linked analogues</u> were studied by <u>in vitro</u> and <u>in vivo</u> assays. <u>Soft agar cloning assays</u> were used to determine <u>cytotoxicity</u> , <u>DNA cross linking to determine biochemical effects</u> , and <u>in vivo cell kill assay to determine biological effects</u> .		

Project Description

Objectives:

To determine whether the interaction of sulfhydryl derivatives of cyclophosphamide and polymeric analogues of these compounds with tissues and cells is altered relative to a phosphoramidate standard in vitro and to cyclophosphamide in vivo.

To investigate whether misonidazole has any synergistic effect on the action of the above alkylating agents and to study the mechanism of their interaction.

Methods and Major Findings:

The sulfhydryl derivatives and their polymer linked analogues are synthesized by Dr. Ringsdorf's group in Mainz, Germany. We have been involved in evaluating their biochemical, toxicological and antineoplastic properties.

Last year we demonstrated that a substantial increase in toxicity was produced by linking the polymeric compound DIVEMA to antitumor agents. DIVEMA is a negatively charged polymer; its immunestimulating characteristics served as the basis for linking it to immunosuppressive antineoplastic agents. Because of greatly enhanced toxicity in vivo we were unable to compare the DIVEMA-linked sulfhydryl derivative of cyclophosphamide to phosphoramidate mustard at equal doses to determine if tumor cells had any degree of preferential uptake of a polyanionic large molecule. In vitro studies this year permitted us to make this comparison. The sulfhydryl derivative was compared to this analogue attached to DIVEMA for cytotoxicity in vitro and DNA damaging effects. The sulfhydryl derivative consistently produced twice the cell kill (soft agar colony formation of L1210) and twice the interstrand DNA crosslinks (alkaline elution assay) as did an equivalent amount of sulfhydryl derivative attached to DIVEMA.

These data clearly indicate that the attachment of a low molecular weight antineoplastic agent to a highly negatively charged polymer can substantially alter the interaction of the agent with tissues. It appears that polyanionic polymers are selective for normal tissues rather than tumor cells. This concept may be exploited with certain protective agents.

The biological effects of synthetic polymers with a variety of charge densities are being explored. Polyethyleneimine N-2 carbonylethyl-ethyleneimine copolymers with various degrees of reduced negative charges have been attached to the same sulfhydryl cyclophosphamide derivatives. Preliminary results indicate that these compounds are less toxic and more active against L1210 in vivo than their DIVEMA analogues. Further exploration is warranted when more compound becomes available.

It has been suggested that misonidazole, in addition to acting as a radiosensitizer, may also sensitize cells to the action of alkylating agents. Since we have studied DNA crosslinking and repair following exposure to the sulfhydryl derivatives of the alkylating agent cyclophosphamide, it seemed appropriate to determine whether enhanced crosslinking of DNA or inhibition of repair was

responsible for synergistic effects of misonidazole with alkylators. Preliminary results indicate that misonidazole interferes with DNA repair processes. In addition, we have observed that anaerobic incubation conditions appear to interfere with the catabolism of the alkylating species to non-active components, by anaerobic inhibition of aldehyde dehydrogenase; the inhibition enhances the toxicity of sulfhydryl alkylators by several fold. This implies that cyclophosphamide-like drugs may be more effective in anaerobic than in aerobic environments.

Publications:

1. Hirano, T., Ringsdorf, H., and Zaharko, D. Antitumor activity of monomeric and polymeric cyclophosphamide derivatives compared with in vitro hydrolysis. Cancer Res. 40: 2263-2267, 1980.
2. Ramonas, L. M., Erickson, L. C., Ringsdorf, H. and Zaharko, D. S. Effect of dose, schedule and route of administration on the in vivo toxicity and antitumor activity of two activated sulfhydryl derivatives of cyclophosphamide. Cancer Res. 40: 3704-3708, 1980.
3. Erickson, L. C., Ramonas, L. M., Zaharko, D. S., and Kohn, K. W. Cytotoxicity and DNA crosslinking activity of 4-sulfidocyclophosphamide in mouse leukemia cells in vitro. Cancer Res. 40: 4216-4220, 1980.
4. Ramonas, L. M., Erickson, L. C., Klesse, W., Kohn, K. W., and Zaharko, D. S. Differential cytotoxicity and DNA crosslinking produced by polymeric and monomeric activated analogs of cyclophosphamide in mouse L1210 leukemia cells. Mol. Pharmacol. 19: 331-337, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06142-04 LCHP												
PERIOD COVERED October 1, 1980 to September 30, 1981														
TITLE OF PROJECT (80 characters or less) Studies of thymidine and other nucleosides as modifiers of antineoplastic drug action														
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">D. S. Zaharko</td> <td style="width: 35%;">Head, Pharmacokin. & Pharmacodyn. Sec.</td> <td style="width: 15%;">LCHP NCI</td> </tr> <tr> <td>Other:</td> <td>L. Ramonas</td> <td>Staff Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td></td> <td>E. Herman</td> <td>Scientist</td> <td>DDB FDA</td> </tr> </table>			PI:	D. S. Zaharko	Head, Pharmacokin. & Pharmacodyn. Sec.	LCHP NCI	Other:	L. Ramonas	Staff Fellow	LCHP NCI		E. Herman	Scientist	DDB FDA
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Other:	L. Ramonas	Staff Fellow	LCHP NCI											
	E. Herman	Scientist	DDB FDA											
COOPERATING UNITS (if any) Division of Drug Biology, FDA														
LAB/BRANCH Laboratory of Chemical Pharmacology														
SECTION Pharmacokinetics and Pharmacodynamics Section														
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205														
TOTAL MANYEARS: 0.75	PROFESSIONAL: 0.25	OTHER: 0.5												
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☐ (a1) MINORS ☐ (a2) INTERVIEWS														
SUMMARY OF WORK (200 words or less - underline keywords) HPLC is used to quantitate nucleosides in biological fluids and tissues following administration of <u>thymidine</u> and other <u>chemotherapeutic</u> agents such as <u>MTX</u> and <u>Ara-C</u> . The purpose of these investigations is to understand and relate pharmacokinetic behavior of these molecules to their <u>biochemical</u> and <u>therapeutic</u> or <u>toxic</u> action both <u>in vitro</u> and <u>in vivo</u> .														

Project Description

Objectives:

To develop methods for extraction and quantitation of various nucleosides and nucleotides in biological fluids and tissues.

To determine biochemical modulation effects of thymidine.

Methods and Major Findings:

Collaborative studies with Dr. Herman concerning the cause of lethality of high dose thymidine in mice were completed. A dose response curve for lethal toxicity indicated a LD_{50} of 4700 mg/kg and LD_{10} of 3800 mg/kg given as a single dose. Within 15 minutes after administration of such doses the mean arterial blood pressure and heart rate fell precipitously. Sedation and anuresis were also observed. Experiments on isolated heart preparations indicated no direct action of thymidine on the heart.

We have confirmed that thymidine is a good suppressor of L1210 growth in vitro if adequate concentrations and exposure times are maintained. We have also compared the cytostatic effect (growth inhibition assay) with the cytotoxic effect (inhibition of colony formation) at various concentrations and exposure times. Cytostasis can be achieved at concentrations of thymidine as low as 0.1 mM when growth is monitored during thymidine exposure. However if thymidine is removed by washing and then growth monitored, concentrations approaching 1mM are needed to sustain cytostasis for 120 hours. Pharmacokinetic studies of thymidine in mice indicated that such conditions of concentration and exposure time could be achieved with multiple injections or constant intraperitoneal infusions with tolerable toxicity. Cytotoxic effects in vitro indicated that a three log cell kill could be obtained at in vivo pharmacokinetically achievable concentrations and exposure times. However extensive in vivo trials indicated no measurable cytostatic or cytotoxic effect of thymidine in L1210 leukemia. Since this ineffectiveness could not be explained on a pharmacokinetic basis (e.g., lack of sufficient thymidine concentration and exposure time), we looked for a biochemical explanation.

It is known that thymidine exerts its cytotoxic effect through thymidine-triphosphate's (TTP) inhibitory effect on the enzyme ribonucleotide reductase. In brief, this inhibition leads to depletion of dCTP, a necessary substrate for DNA synthesis. This cytotoxic effect can be reversed if the salvage metabolite, deoxycytidine, is supplied in sufficient quantity. Our finding was that the plasma deoxycytidine concentration of either normal or L1210-bearing mice prior to thymidine treatment was not sufficiently high to explain the lack of effect of thymidine on the growth of L1210 in vivo. However following thymidine treatment of mice both cytidine and deoxycytidine in plasma and spleen rose to concentrations that were capable of reversing thymidine cytostasis or cytotoxicity in vitro.

We are now exploring the question of how thymidine causes a rise in deoxycytidine in the plasma. One approach we will use will be to study the intracellular kinetics of ribonucleotides under various conditions of L1210 tumor growth in vivo.

Ara-C and Thymidine

Resistance of tumors to ara-C is very likely due to an imbalance of the deaminases and kinases that inactivate and activate ara-C. The metabolic product of thymidine, TTP, is a potent inhibitor of cytidine deaminase as well as an inhibitor of pyrimidine kinases and ribonucleotide reductases. We have attempted to determine whether this selective action on certain enzymes may extend to chemotherapeutic selectivity against tumors versus normal tissues when ara-C and thymidine are used in combination.

Our first trials have been with L1210, a tumor which is sensitive to ara-C. Our findings were that thymidine shifts the dose response curve for ara-C toxicity far to the left, i.e. much less ara-C has to be given for equal toxicity to mice when given with thymidine than when given alone. Thymidine given simultaneously with ara-C results in greater toxicity than when there is a delay between thymidine administration and ara-C. In addition, better therapeutic results were obtained when there was a delay between administration of ara-C and of thymidine than when the drugs were given simultaneously. However ara-C given alone at equitoxic doses to the above combination was superior in therapeutic effects against L1210. The timing of this combination is obviously very important and an investigation of intracellular nucleotides may give a better understanding of the biochemical events that are involved in this phenomenon.

Publications:

1. Herman, E. H., Jordan, W. J., Ardalan, B., Zaharko, D. S., Bolten, B.J., and Cooney, D. A. Toxicologic effects of a high dose of thymidine in mice. Toxicol. Appl. Pharmacol. 56: 443-446, 1980.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06146-03 LCHP																		
PERIOD COVERED October 1, 1980 to September 30, 1981																				
TITLE OF PROJECT (80 characters or less) Control of Neoplastic Diseases by Combined Treatment with Immuno Regulating Factors																				
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 30%;">PI: M. A. Chirigos</td> <td style="width: 40%;">Head, Virus & Disease Modification Section</td> <td style="width: 30%;">LCHP NCI</td> </tr> <tr> <td>Other: V. Papademetriou</td> <td>Visiting Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td>A. Bartocci</td> <td>Visiting Fellow</td> <td>LCHP NCI</td> </tr> <tr> <td>J. Weiss</td> <td>Armed Forces Radiobiological Research Institute</td> <td></td> </tr> <tr> <td>T. K. Steel</td> <td>Armed Forces Radiobiological Research Institute</td> <td></td> </tr> <tr> <td>H. B. Levy</td> <td>Lab. of Viral Disease</td> <td>NIAID</td> </tr> </table>			PI: M. A. Chirigos	Head, Virus & Disease Modification Section	LCHP NCI	Other: V. Papademetriou	Visiting Fellow	LCHP NCI	A. Bartocci	Visiting Fellow	LCHP NCI	J. Weiss	Armed Forces Radiobiological Research Institute		T. K. Steel	Armed Forces Radiobiological Research Institute		H. B. Levy	Lab. of Viral Disease	NIAID
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TOTAL MANYEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0																		
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																				
SUMMARY OF WORK (200 words or less - underline keywords) TPA treatment of <u>macrophages (MΦ)</u> results in <u>prostaglandin (PG)E₂</u> production and a <u>soluble factor(s)</u> promoting tumor cell growth. <u>Interferon(IF)</u> and IF inducers enhance <u>MΦ</u> and <u>Natural Killer (NK)</u> cell <u>tumoricidal activity</u> which is negated by PG. The ability of tumors to produce PGE may contribute to their ability to escape immune surveillance. Glucocorticoid hormones, PG, and cAMP inhibit IF induced <u>MΦ</u> tumoricidal activity. Maleic divinyl ether(MVE) of various molecular weights enhance <u>MΦ</u> and NK cell tumoricidal activity. A synergistic anti-tumor therapeutic effect is achieved by combining MVE with cytoreductive therapy. Human chorionic gonadotropin (HCG) expresses a dual effect: it enhances <u>MΦ</u> and NK cell tumor lytic activity and PG formation which, in turn, limits T cell effects. High titers of IF induced by Poly ICLC correlate with the enhanced <u>MΦ</u> and NK cell tumor lytic effect. Azimexon treatment resulted in increased <u>granulocyte-MΦ colony forming cells</u> in spleen & bone marrow. Highly effective therapeutic response was achieved against an alveolar Ca with combined cytoreductive chemotherapy and MVE. MVEs' effective adjuvantcity to tumor cell <u>vaccines</u> appear to act through the <u>augmentation of MΦ antigen processing</u> .																				

Project Description:Objectives:

The focus of this project is to develop and implement experimental tumor systems (spontaneous, virus-induced or carcinogen-induced) for investigating combined modality treatments (immunoadjuvants combined with chemotherapy, radiation and/or surgery) with the aim of improving therapy of human malignancies. Studies are conducted on immunologic and pharmacological mechanisms by which immunoadjuvants activate and stimulate the various cellular components (T cells, B cells, macrophages, granulocytes) of the immune system. Diagnostic tests are developed for monitoring the effect of immunoadjuvant therapy which influences tumor regression. Immunoadjuvants are also evaluated for their potential use in tumor cell vaccines.

Methods Employed:

- A. Established or newly developed animal tumors are employed for in vitro and in vivo studies.
- B. Immune responses, both humoral and cellular, are measured by standard procedures or by new methods developed by Section staff. These assays include T and B cell mitogens, T and B cell immunofluorescent antibody, macrophage and lymphocyte cytotoxicity tests, T cell rosette, skin grafts and allogenic tumor grafts, natural killer cell activity, and delayed type hypersensitivity.

Major Findings:

TPA (12-O-tetradecanoylphorbol-13-acetate), the potent inflammatory and tumor promoting agent was investigated in vitro and in vivo. In vitro TPA treatment of macrophages at 1.0ng/ml induced prostaglandin E_2 release and morphological changes analogous to cell activation. Of particular interest, conditioned medium from macrophages pulsed with TPA enhanced M109 carcinoma colony formation in vitro. Macrophages were not rendered tumoricidal by TPA in vitro; cytotoxic macrophages were recovered from mice following ip treatment with TPA at concentrations of 1-100 μ g/kg indicating an indirect pathway for the activation of macrophages by TPA. The very weak tumor promoting 4-O-methyl-12-O-tetradecanoylphorbol-13-acetate lacked effects on macrophages. The possibility exists that macrophage secretions (e.g. prostaglandin E_2 , angiogenesis-stimulating factor(s), and clonal proliferation factor(s) for carcinogen-triggered cells) may be involved in the tumor promotion process.

Normal mouse macrophages are activated (rendered nonspecifically cytotoxic) by treatment with chromatography-purified, virus-induced fibroblast interferon. Prostaglandins of the E-series (PGE), administered in vitro or in vivo, suppressed the cytotoxicity of interferon-treated macrophages for MBL-2 lymphoblastic leukemia cells in a dose-dependent fashion. E-type prostaglandins apparently suppress macrophage function by enhancing intracellular levels of cyclic AMP. Treatment with the macrophage activator, endotoxic lipopolysaccharide, but not partially-purified interferon, stimulated macrophages to produce PGE, indicating that the 2 macrophage functions, cytotoxicity and PGE secretion, completely dissociate. Moreover, prostaglandin production by macro-

phages following certain activation stimuli could allow for negative feedback inhibition to limit cell activities. The ability of tumors to produce E-type prostaglandins may be an important contributing factor by which they escape surveillance by activated macrophages. The tumoricidal activity of interferon-activated macrophages can be modulated *in vitro* by low concentrations of various pharmacologically active molecules. Glucocorticoid hormones and their synthetic derivatives, as well as E-type prostaglandins, cholera toxin and dibutyryl cyclic AMP, markedly inhibit the cytotoxic activity of interferon-treated macrophages for MBL-2 leukemia cells, even when applied after the macrophages have reached full morphologic activation. The effect of glucocorticosteroids is of particular interest, both because their relative anti-inflammatory potencies correlate with their ability to inhibit macrophage function in culture and because this effect occurs at near physiologic concentrations. We have also examined the ability of peritoneal macrophages from mice, which were stressed by physical restraint, to respond to activation stimuli *in vitro* and *in vivo*. Macrophages from these mice showed a depressed functional activity which is postulated to have resulted from elevated plasma corticosteroid levels. The results indicate that activated macrophage tumor cytotoxicity is not a highly determined event, but rather a relative capability regulated by several factors in the local environment of the macrophage.

The effects of exogenously added prostaglandin (PG) E_1 or E_2 over concentration ranges of from 1×10^{-4} to 1×10^{-9} M were studied in order to determine their effect on the delayed-type hypersensitivity (DTH) reaction of normal or tumored mice. PGE_1 or PGE_2 generally caused a stimulation over the control values of normal mice as detected by the footpad swelling assay. However, PGE_1 or PGE_2 at all concentrations tested were found to significantly inhibit the DTH reaction of CD_2F_1 tumored mice.

A series of maleic anhydride divinyl ether (MVE) polyanions, synthesized with molecular weight ranging from 12500 to 52600, were found capable of enhancing macrophage tumoricidal activity against MBL-2 leukemia cells. These agents also augmented Natural Killer cell activity against the YAC lymphoma and M109 adenocarcinoma cell lines. This response appears to be dependent upon MVEs ability of activating macrophage tumoricidal activity. When these agents were combined with Cytoxan cytoreductive therapy an enhanced antitumor (curative) response was achieved. The remarkable combined effect may be attributable to the effective tumor cytoreductive response to Cytoxan followed by an augmented immunological response by MVE through the action of activated macrophage tumoricidal effect on residual tumor cells.

The fetus during normal pregnancy is regarded as an intrauterine transplant but instead of the expected rejection, a parasitic graft-host relationship is established. This relation is similar to that of an autologous tumor which can circumvent host immune factors and grow progressively. Since the human chorionic gonadotropin (HCG) is considered an absolute requirement for successful continuation of pregnancy and since experimental evidence indicates that HCG is immunosuppressive, studies were conducted to determine what effect HCG had on natural killer cell tumoricidal activity.

The natural killer (NK) activity, of an allogeneic tumor system, was found to be enhanced by the total splenic population of either pregnant or normal female

BALB/c mice treated with human chorionic gonadotropin (CG). The splenic population of the early pregnant (9-10 days) mice resulted in a statistically significant increase in NK activity over nonpregnant controls at all effector/target ratios tested. The mid (12-13 days) and the late (15-16 days) pregnant groups also significantly enhanced NK activity above control values at effector/target ratios of 100:1, 50:1 and 25:1. The i.v. administration of carrageenan into pregnant mice abolished the increased NK activity suggesting a putative role for the macrophage in this system. HCG administered to normal female BALB/c mice resulted in enhanced NK activity similar to that seen for the pregnant mice. This augmented NK activity does not appear to be affected by either the number or the timing of HCG administration. The augmentation of NK activity of HCG treated mice was also detected in a syngeneic system utilizing ^{51}Cr labeled M109 tumor target cells and BALB/c effector cells. Although HCG has been suggested to possess immunosuppressive properties, it was interesting to note that the NK cell, which is a cellular component of the immune system, was enhanced in its activity by HCG. Further studies were conducted to assess the effect of HCG on another cell of the immune system, the macrophage. *In vitro* concentrations higher than 100 IU/ml were found to render macrophages cytotoxic against MBL-2 leukemia cells. Daily intraperitoneal injections of 500 IU/mouse for a total of six or more treatments also rendered peritoneal macrophages cytotoxic. The *in vivo* HCG-activated macrophages were found to be suppressive for normal splenic lymphocytes at lymphocyte: macrophage ratios of 10:1 to 2.5:1. *In vitro* treatment of macrophages with HCG from 100 to 2500 IU/ml resulted in elaboration and release of high concentrations of prostaglandins, notably of the E series. The results of this study strongly suggest that some of the previously reported immunosuppressive activities of crude HCG may be mediated by macrophages through prostaglandin production.

Poly-ICLC, a polyinosinic:polycytidylic acid stabilized with poly-L-lysine in carboxymethylcellulose, was tested in mice for its immunoregulatory activity. Poly-ICLC was found to enhance T cell responsiveness but not B cells. It augmented the delayed type hypersensitivity response significantly. The results indicate Poly-ICLC to be a T cell stimulator. Macrophage tumoricidal activity was markedly enhanced both *in vitro* and *in vivo* after exposure to Poly-ICLC. Natural killer cell cytotoxicity was significantly augmented *in vivo*. Both macrophage and natural killer cell activity was maintained for over 3 days after only one treatment. The extended period of tumor cell cytotoxicity exhibited by macrophages and natural killer cells may correlate with Poly-ICLC induction of early and high levels of interferon which are maintained in the serum for a longer period of time.

The clonal proliferation of the committed granulocyte macrophage stem cell was examined in the bone marrow (BM) from pregnant and normal female mice in the presence of Colony Stimulating Factor (CSF). Three different stages of pregnancy were examined and an increased number of Colony Forming Unit Cells (CFU-C) was observed for all the pregnancy groups relative to the non-pregnant female control. The enhancement in the proliferative status of the precursors was accompanied by a decrease in the total BM nucleated cells and by a rise in the number of macrophages in the peritoneal cavity. In addition, a marked increase of prostaglandin production by peritoneal macrophages was detected in pregnant hosts. The placental production of CSF is proposed to be a mediator of the physiological changes found in the present study.

Azimexon (BM 12.531) which we reported last year to be effective in reconstituting the cell population in mice receiving total body irradiation or cytoreductive chemotherapy, was also examined for its effect on mouse granulocyte-macrophage & monocyte-macrophage progenitor cells. Treatment of mice with 25mg/kg Azimexon resulted in an increase in granulocyte-macrophage colony-forming cells in spleen and bone marrow after a transient depression in the cell populations. Bone marrow monocyte-macrophage colony forming cells (MM-CFC) increased at 7 days after treatment, and splenic MM-CFC were least affected by Azimexon treatment. The increase in granulocytic and monocytic colony-forming cells may play a role in the previously reported protection by Azimexon against radiation and drug induced toxicity.

The M109 alveolar carcinoma, a tumor which is refractory to several chemotherapeutic agents, was employed to assess the effect of combined chemo-immunotherapy. A marked reduction of lung tumor lesions was achieved, resulting in a longer remission period and a significant number of long term survivors, with combined BCNU chemotherapy and MVE2 immunotherapy. Ancillary studies indicate that this treatment was successful because the primary chemotherapy reduced the tumor burden sufficiently so that tumoricidal macrophages, activated by the secondary MVE2 treatment, further reduced residual tumor cells in the lung.

The maleic divinyl ethers were examined further for their capacity to act as adjuvants to tumor cell vaccines. Augmentation of the immune response was achieved when the MVE's were combined with irradiation killed vaccines of L1210, LSTRA (leukemias), and the B16 melanoma. Protection against live tumor cell challenge was found to be tumor cell specific. No cross-protection was achieved. Macrophage involvement in antigen processing of the tumor cell vaccine was demonstrated when carrageenan, a macrophage inactivator, was shown to abort the protective effect obtained with vaccination and MVE treatment. B cell participation was considered obligatory since vaccinated mice, surviving live tumor cell challenge, were also shown to reject a second live tumor cell challenge several weeks later.

Significance to Biomedical Research and the Program of the Institute:

The multidisciplinary approach represented by this project is entirely directed towards the prevention, treatment and control of cancer. Studies with combined immunotherapeutic modalities have led to a more effective control of neoplasia without an additional burden to the host beyond that imposed by chemotherapy. The advantages of such combined modes of treatment are: (1) no additional cytoreductive chemotherapy is needed to maintain remission; (2) the immune competence of the host is restored allowing host immune mechanisms to function more effectively; (3) maintenance of remission may be obtained by intermittent treatment with immunoadjuvants which lack the limitations inherent with continuous cytoreductive chemotherapy; (4) refractoriness to immunoadjuvants, if it does develop, can be supplemented by additional chemotherapy with the advantage that the host is in a better physical condition to receive subsequent chemotherapy.

Experimental studies conducted with chemical (levamisole, Azimexon, poly ICLC, cimetidine, etc.) and biological (interferon, BCG, thymosin fractions, levan, lentinan, glucan, etc.) agents in respect to their immunoadjuvant effects, most

efficacious treatment regimens, and usefulness in combined treatment modalities, has provided information leading to the inclusion of some of these agents for the treatment of human breast, colon and rectal carcinomas, head and neck tumors, and leukemias. This section is, and will continue, collaborating with participating cancer treatment centers requesting information concerning any of the tested agents.

The ability of several immune modulators to specifically and strongly augment host immunity when they are used alone or in concert with established cancer treatment modalities is of practical value in preventing and/or controlling cancer. Basic research studies conducted with these immunoregulatory agents are defining the cellular components which are activated by these agents and the specificity of their tumoricidal activity.

The ability of the adjuvants to specifically and strongly augment antitumor immunity of isologous tumors after vaccination is of practical value. Of particular significance would be the finding that soluble tumor cell antigens, rather than irradiated tumor cells, can be used for vaccination in concert with the chemical adjuvants. This preventive approach will be applicable to groups at high risk for specific tumor types.

Proposed Course:

Each of the areas described under Objectives will be pursued. Emphasis will be placed on quantitative and qualitative studies in three specific areas, i.e., immunostimulating adjuvants, combined treatment modalities, and tumor vaccine studies. The role of immunoadjuvants in protracting the remission period induced by chemotherapy in leukemias and surgery in solid tumors will be investigated in detail. Additional in vitro and in vivo methods will be implemented to further define the mechanisms of action of the active chemical and biological immunostimulators in terms of T cell, B cell, natural killer cell or macrophage function.

Since the macrophage and natural killer cell are emerging as important host tumoricidal cells studies will be continued in determining the mechanisms of their enhancement. In vivo studies will be conducted to ascertain the most efficient method of combining tumor cytoreductive therapy with agents that protect and/or augment macrophage and natural killer cell activity, with the aim of improving present cancer therapeutic modalities. Studies will be pursued to assess the role that prostaglandins play in the regulation of the cellular immune response. A continuing effort will be made to identify new and more effective immune augmentors and define their mechanism of action.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06148-02 LCHP														
PERIOD COVERED October 1, 1980 to September 30, 1981																
TITLE OF PROJECT (80 characters or less) Endogenous Modifiers of Drug Action																
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 35%;">Richard L. Cysyk</td> <td style="width: 35%;">Head, Drug Metabolism Section</td> <td style="width: 15%;">LCPH NCI</td> </tr> <tr> <td rowspan="3">Other:</td> <td>Jean M. Karle</td> <td>Staff Fellow</td> <td>LCPH NCI</td> </tr> <tr> <td>Patricia A. Monks</td> <td>Visiting Fellow</td> <td>LCPH NCI</td> </tr> <tr> <td>John M. Strong</td> <td>Sr. Staff Fellow</td> <td>LCPH NCI</td> </tr> </table>			PI:	Richard L. Cysyk	Head, Drug Metabolism Section	LCPH NCI	Other:	Jean M. Karle	Staff Fellow	LCPH NCI	Patricia A. Monks	Visiting Fellow	LCPH NCI	John M. Strong	Sr. Staff Fellow	LCPH NCI
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																
SUMMARY OF WORK (200 words or less - underline keywords) Studies were continued on the biologic importance of <u>circulating uridine</u> and the relative importance of the <u>de novo</u> and salvage pathways for uridine nucleotide biosynthesis. The role of the <u>liver</u> as a source and modulator of serum uridine concentrations was investigated using an <u>isolated perfused rat liver system</u> . Studies were initiated using <u>isolated rat hepatocytes</u> to obtain biochemical information on the regulation of pyrimidine biosynthesis. Studies on the disposition of <u>C¹⁴-uridine</u> in rats were begun. An <u>in vitro</u> system was developed to maintain constant concentrations of uridine in cell cultures. Concentrations of uridine equivalent to that measured in mouse sera were found to alter the cell growth inhibitory action of PALA and to alter the flux through the <u>de novo</u> pyrimidine pathway. A new GC-mass spectrometric technique for <u>uridine quantitation</u> was developed and the flux through the <u>de novo</u> pyrimidine pathway was determined in cultured cells using this assay in conjunction with <u>stable isotopes</u> .																

Project Description

The activity of certain antimetabolite antitumor agents is the result of their action as enzyme inhibitors. The cytotoxic and antitumor effects of these agents are known to be influenced by administration of the product of the inhibited reaction or by compounds that can circumvent the inhibited reaction. For example, inhibition of the de novo pyrimidine biosynthetic pathway can be overcome by administration of preformed pyrimidines that can be utilized via salvage pathways. The overall objective of this project is to determine if antitumor agents alter the availability (via the serum) of endogenous compounds that have the potential of influencing the toxic effects of the agent at the tumor site.

Specifically:

1. To determine if inhibitors of the de novo pyrimidine and purine biosynthetic pathways alter circulating levels of preformed nucleosides that have the potential of reversing the cytotoxic effects of these agents at the tumor site.
2. To determine the mechanism of regulation of serum levels of pyrimidine and purines and if these levels can be altered to influence drug effect.
3. To determine if it is necessary to inhibit de novo synthesis in normal donor organ(s) simultaneously with inhibition in tumor cells in order to achieve an antitumor effect.
4. To determine if drug-induced fluctuations in serum levels of endogenous nucleosides can be used to rationally design scheduling and combinations of drugs for maximum chemotherapeutic advantage.

Methods Employed:

Usual biologic, pharmacologic and biochemical techniques. Among these are analytical techniques for quantitating endogenous compounds; isolated organ perfusion techniques; tissue culture techniques; tumor transplantation and harvesting of cells; enzyme isolation; techniques for measuring the flux through biosynthetic pathways.

Major Findings:Studies on the plasma kinetics of uridine in rats.

A study was initiated to examine the bioregulation of circulating uridine. [¹⁴C]Uridine was injected into the femoral vein of Sprague-Dawley rats and samples of blood were collected at timed intervals via a cannula in the abdominal aorta. The resulting rapid decrease of radioactive uridine in the collected blood samples demonstrated that uridine exhibits a half-life in the blood in the range of 1-4 minutes. When labelled uridine is injected along with 1000-10,000 nmol of unlabelled uridine (normal rat plasma uridine levels are 1.5-4.7 nmol/ml), plasma uridine levels rapidly return to normal, and the half life of

circulating [^{14}C]uridine remains in the range of minutes. Two inhibitors of nucleoside membrane transport, dipyridamole and nitrobenzylthioinosine 5'-phosphate (NBMPR-P), were injected prior to [^{14}C]uridine. Neither compound appeared to affect the kinetics of circulating uridine.

Regulation of circulating uridine by the isolated rat liver.

Uridine has been shown to reduce the toxicity of inhibitors of pyrimidine *de novo* biosynthesis, such as pyrazofurin and N-(phosphonacetyl)-L-aspartate. Since plasma is an available source of uridine *in vivo*, we have investigated the role of the isolated liver as a source and/or regulatory mechanism of circulating concentrations of uridine. We have utilized the isolated rat liver for these studies and evaluated a synthetic blood substitute, Fluosol 43, as a perfusion medium. The advantage of a synthetic medium for these studies is that it contains no substrates or enzyme activities which would be present in a simplified blood preparation. We have concluded that Fluosol 43 is a good, inert, perfusion medium for our purposes which could supply oxygen to the active liver cells.

The isolated rat liver excreted uridine into a circulating perfusion medium achieving concentrations similar to those found in rat plasma ($1.4 \pm 0.6\text{mM}$). The mean output of uridine over 2h was 107nmol/h/g of liver, and was not the result of cell necrosis as no nucleotides were found in the perfusate. The excretion of uridine requires enzymatic degradation of uridine monophosphate by hepatic 5'-nucleotidase, as the free nucleoside is not stored in the liver. The rate of excretion of uridine by the liver responds to changes in the circulating concentrations of uridine. A 10 fold decrease in output (from 107 to 12.7nmol/h/g over 2h) was observed when the excreted uridine was allowed to increase until an equilibrium was reached at approximately $1\mu\text{M}$. The rate of depletion of uridine concentrations in excess of plasma levels was concentration dependent and found to be linear in the range of $1\text{--}25\mu\text{M}$.

At a uridine concentration of approximately $1\mu\text{M}$, there is no net change in the circulating concentration; the uptake and export of uridine are balanced and at equilibrium. At this steady state concentration, a single radioactive uridine spike is rapidly lost from the perfusate by an apparent first order process, with a half life of 7.4 min. This elimination is apparently due mainly to the rapid distribution of the radioactive uridine into the uracil nucleotide pool of the perfused liver since the specific activity of the circulating uridine was reduced by a factor equivalent to the concentration of total hepatic uracil nucleotides. There was also catabolism of the circulating uridine, as evidenced by chromatographic analysis of the perfusate. Uridine is stored in the liver as the nucleotide, not the nucleoside, and therefore the equilibrium concentration of approximately $1\mu\text{M}$ achieved in the plasma reflects a balance between uridine kinase and 5'-nucleotidase activity.

Further studies intended for this model will involve investigation of purine regulation by the liver and effect of certain inhibitors on the influx and efflux of purines and pyrimidines in the isolated liver.

Flux through the de novo pyrimidine biosynthetic pathway in isolated hepatocytes.

To facilitate biochemical investigation of pyrimidine regulatory activity of the liver, we initiated studies utilizing isolated rat hepatocytes for tracing [^{14} C]-bicarbonate incorporation into the uridine nucleotide pool of these cells. In media without added ammonia, the rate of de novo synthesis is minimal, with only 7% of the available bicarbonate being incorporated into the nucleotide pool. In the presence of 0.7mM (physiological concentrations) of ammonium chloride, the rate and amount of incorporation was vastly stimulated until after 2h, 50% of the available bicarbonate could be accounted for in the uridine pool. The size of this pool however was not expanded in the presence of 0.7mM NH_4Cl , nor when the NH_4Cl concentration was increased to almost toxic levels of 5mM. Additionally the rate of de novo synthesis did not appear to be significantly increased by the higher concentration of ammonium chloride, the incorporation at 2h being only 60%.

Effect of serum concentrations of uridine on the growth inhibitory effect of PALA in vitro.

Cells that contain uridine/cytidine kinase have the ability to utilize extracellular uridine in order to circumvent inhibition of the de novo pyrimidine biosynthetic pathway. We became interested in the capability of uridine at normal serum levels for humans, rats, and mice (2-12 μM) to reverse the cell growth inhibitory effects of PALA, an inhibitor of de novo pyrimidine biosynthesis, in tissue culture. Since both L1210 cells and Lewis lung carcinoma cells rapidly deplete uridine from cell culture media, a method for infusing uridine was devised in order to maintain the media uridine at constant levels. At an initial concentration of 100 μM , L1210 cells depleted media uridine at a rate of 0.3nmol/hr/10⁵ cells and Lewis lung carcinoma cells depleted media uridine at a rate of 7 nmol/hr/10⁵ cells. All media uridine levels were quantitated by HPLC. When the medium was made 100 μM in PALA and the media uridine levels maintained between 4-18, 4-8, 1-3, and 0.3-1.2 μM by constant infusion, L1210 cell growth at 72 h was 81, 70, 55, and 27% of untreated uridine-infused controls, respectively. L1210 cell cultures which were made 100 μM in PALA, but were not infused, grew to 13, 21, 8, and 14% of control, respectively, at 72 hr. PALA treated L1210 cells required 10-20% more infused uridine to maintain the media uridine level equal to that of untreated, infused cell cultures. When the media of Lewis lung cells was made 50 μM in PALA and the media uridine level was maintained between 3-0, 3.5-6.5, 0.5-2.0, and 0.4-2.5 μM , cell growth at 48 hr was 80, 71, 67, and 37% of untreated, uridine infused controls, respectively. Lewis lung cell cultures which were made 50 μM in PALA, but were not infused with uridine, grew to 22, 15, 35, and 4% of controls at 48 hr, respectively. PALA treated Lewis lung cells required 5-10% more infused uridine to maintain the media uridine level equal to that of untreated, infused cultures. Since extracellular uridine at normal serum levels has the capability to greatly reduce the growth inhibitory effects of PALA on L1210 and Lewis lung cells in vitro, serum uridine may be reducing the antitumor effectiveness of inhibitors of the de novo pyrimidine biosynthetic pathway in vivo.

Effect of uridine on the flux through the de novo pyrimidine biosynthetic pathway of L1210 cells in vitro

A new study was initiated to examine the relative use of de novo pyrimidine biosynthesis versus salvage of pyrimidine nucleotides by cells in tissue culture. High concentrations of uridine ($> 100\mu\text{M}$) have been shown to inhibit incorporation of labelled bicarbonate into the uracil nucleotides of cells in culture. However, these uridine concentrations are well above normal serum levels ($2\text{--}12\mu\text{M}$). Since L1210 cells rapidly deplete uridine from culture media (at 10^6 cells/ml, rate of uridine depletion is $11\text{--}14\text{ nmol/hr/ml}$), a method for infusing uridine into tubes incubating in a shaking water bath was devised in order to maintain constant uridine levels. Preliminary results indicate that the incorporation of $[^{14}\text{C}]\text{HCO}_3^-$ into the uracil nucleotides of L1210 cells after 1 hr is 14% of controls when the media uridine levels are maintained between $0.3\text{--}2.0\mu\text{M}$. At media uridine levels of $10\text{--}12.5\mu\text{M}$, bicarbonate incorporation is reduced to less than 3% of controls. These results indicate that at normal serum levels L1210 cells depend mainly on extracellular pyrimidines rather than newly synthesized pyrimidines to maintain their pyrimidine pools. If this is also the case for tumor cells in vivo, then the lack of therapeutic response by humans towards inhibitors of de novo pyrimidine biosynthesis may be explained by a reduced dependency of tumor cells on the de novo pyrimidine biosynthetic pathway.

Effect of chemotherapeutic agents and transport inhibitors on circulating concentrations of uridine in BDF₁ mice

Since BDF₁ mice possess a relatively constant concentration of serum uridine ($9.7\text{ nmol/ml} \pm 1.3\text{ S.D.}$), circulating uridine is available to cells with an intact pyrimidine salvage pathway and thus could influence the effectiveness of certain antitumor agents which inhibit de novo pyrimidine biosynthesis and whose cytotoxic properties are reversed by uridine. Three inhibitors of the de novo pyrimidine biosynthetic pathway were studied to determine their effects on circulating uridine concentrations in BDF₁ mice. Pyrazofurin and 6-azauridine were found to have no significant effect on serum uridine levels when administered as a single dose or on 4 consecutive days. In contrast, N-(phosphonacetyl)-L-aspartate reduced serum uridine levels by 55% when administered either as a single dose or on 4 consecutive days. This reduction could contribute to the antitumor effectiveness of N-(phosphonacetyl)-L-aspartate by limiting the rescue of cells possessing a salvage pathway. Although serum uridine levels in patients treated with PALA were also found to decrease from predose serum levels, they generally remained in the range of serum levels for normal humans. However, mouse serum uridine levels after PALA treatment consistently fell to about half of the normal range which may help to explain, in part, why PALA is curative towards Lewis lung carcinoma cells in BDF₁ mice but is ineffective in humans.

A preliminary study of the effects of dipyridamole and NBMPR-P on BDF₁ mouse serum uridine levels was begun. These drugs which act as inhibitors of nucleoside membrane transport have the potential for disrupting the normal bioregulation of serum uridine. Dipyridamole injected ip (2000 mg/kg in 0.5% CMC) had

little effect on serum uridine levels at 1,2,4,8, and 24 hr. However, when dipyridamole was co-injected with PALA (200mg/kg), serum uridine levels dropped only slightly (30%) at 24 hr and returned to normal at 48 hr. PALA alone consistently causes a 55% drop in serum uridine from 24-96 hr. NBMPR-P at 25 mg/kg (dissolved in H₂O) injected i.p. resulted in normal to slightly above normal (25%) serum uridine levels from 1-8 hr. However, when NBMPR-P was co-injected with PALA (200 mg/kg), elevated serum uridine levels were observed from 1-8 hr which in some cases were > 2x normal. By 24 hr, serum uridine had fallen to levels below normal which is consistent with the 4 hr half life in vivo of NBMPR-P observed by other researchers.

Measurement of flux through the de novo pyrimidine biosynthetic pathway using staple isotope incorporation

We developed a gas chromatographic-mass spectrometric (GC-MS) method for measurement of the incorporation of a stable isotopically labelled precursor of the de novo pyrimidine biosynthetic pathway into the acid soluble uracil nucleotide pool of L1210 cells. The method developed for measurement of [¹³C]-bicarbonate incorporation into the nucleotide pool of L1210 cells in vitro was compared with the standard technique of incorporation of radioactively labelled bicarbonate into the same cells. The total uracil nucleotide pool from the cells was degraded enzymatically to uridine which was the actual compound measured. GC-MS analysis of uridine required its derivatization to a more volatile state. The chemical derivatization involved permethylation of uridine by methyl iodide in the presence of a strong base. The permethylated uridine was analyzed on a HP5710 gas chromatograph linked via a membrane separator to an HP5980A quadropole mass spectrometer with an HP5933A data system. Selected ions were monitored, and the ratio of [¹³C]/[¹²C]-uridine was calculated from the relative abundance of the ion fragment of mass 170 representing the [¹³C] complement compared to the ion fragment of mass 169 (C₇H₉N₂O₃) representing the [¹²C] content. A 199 ion fragment (C₈H₁₁N₂O₄) was additionally monitored to ensure maintenance of a constant ratio between the 2 fragments from the [¹²C]-uridine.

The incorporation of the stable and radioactive isotopes into the acid soluble uridine nucleotide pool of L1210 cells was the same, indicating that our GC-MS method was analogous to the more common tracer technique which uses radio-labelled precursors. The results from this study gave evidence of compartmentalization of uridine nucleotides in L1210 cells. The rate of bicarbonate incorporation was linear for only the initial 20 minutes and a maximum of only 50-60% of the nucleotide pool could be labelled. This indicated that de novo synthesis supplies only half of the total nucleotides and these newly synthesized nucleotides may be utilized by specific RNA species. The remaining nucleotides in the pool must be supplied by salvage and reutilization of breakdown products.

We are currently attempting to use the stable isotope incorporation technique to measure the flux through the de novo pyrimidine biosynthetic pathway in vivo.

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COOPERATING UNITS (if any) Lab. of Biorganic Chem., NIAMDD; Lab. of Exp. Path., Lab. of Med. Chem. and Biol., and Lab. of Viral Carcinogenesis, NCI; Depts. of Medicine and Radiology and Anatomy, University of Calif., San Francisco; Dept. of Pharmacology, Harvard Medical School, Boston																																			
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SUMMARY OF WORK (200 words or less - underline keywords) Various drug-induced morphological changes in animals and man have been reported for a wide variety of agents. Descriptions of severe changes, more appropriately termed toxicities, have been quite valuable in clinical areas (for the prediction of dose-limiting toxicological problems) and in drug development areas (for evaluation of the relative efficacy of analogs). However, such purely descriptive approaches to drug-induced morphological changes are inadequate. They may not help predict undesirable side effects, and often they do not suggest how to more safely utilize a drug. Therefore, it has become necessary to study the mechanisms by which drugs induce morphological changes as means of better understanding their actions. Principal areas of effort are currently being directed toward understanding the following: 1) the mechanisms by which <u>anthracyclines</u> are cardiotoxic; 2) the mechanisms by which <u>platinum compounds</u> are toxic; and 3) how chemotherapeutic agents and tumor promoters affect <u>natural killer cells</u> and other cell lines in tissue culture.																																			

Project DescriptionMethods Employed:

Standard techniques of morphological and biochemical analysis have been employed in these studies.

Objectives:

To follow morphological leads and use morphological and biochemical approaches to investigate the mechanism by which various antitumor agents can induce toxicities. Such information should be of value in 1) designing new, less toxic, analogs of currently useful oncolytic agents, and 2) developing clinical protocols that minimize toxicological manifestations of antitumor agents.

Major Findings:A. In Vitro Cardiotoxicity Testing of New Oncolytic Agents

Utilizing a rat cardiac myocyte system developed in our laboratory, we have tested the cardiotoxic potential of several anthracyclines, two anthracenediones, and m-AMSA. Interestingly, m-AMSA is toxic to the beating cells in a manner very similar to that of adriamycin, but the anthracenediones were not toxic at the limits of their solubilities (greater than 10^{-6} M). Two anthracyclines, AD-32 and aclacinomycin A, were not toxic at the maximal concentrations tested. They were also the only two anthracyclines evaluated that did not appear to bind to nuclei of the heart cells as evaluated in viable cells with the fluorescence microscope.

B. Toxicity of cis-Dichlorodiammineplatinum II (cis-DDP, Cisplatin)

cis-DDP is an effective anticancer agent against embryonal carcinoma and epidermoid carcinomas in man. cis-DDP is also associated with toxicity involving kidney, intestine, and bone marrow. To understand the mechanism of toxicity of cis-DDP, we have studied the cellular interactions of cis-DDP in kidney, intestine and spleen based on morphological, biochemical and functional parameters.

1. Renal Toxicity

Cisplatin nephropathy and renal accumulation of platinum were analyzed in rats after acute and chronic treatment. A single i.p. dose of cisplatin (6 mg/kg) induced marked focal necrosis in the proximal and distal tubules with a maximum lesion on day 7. The tubular damage was localized mainly in the corticomedullary region, where the concentration of platinum was the highest within the kidney. Repeated treatment with cisplatin (1 mg/kg, i.p., twice weekly) for 11 weeks resulted in massive tubular dilation in the corticomedullary region, interstitial fibrosis and thickening of tubular basement membranes. Some glomeruli appeared fibrotic, indicating that chronic treatment with cisplatin could cause irreversible renal damage.

2. Intestinal Toxicity

Intestinal cytotoxicity of cisplatin was qualitatively and quantitatively characterized in rats following a single ip dose (5 mg/kg). Cellular necrosis and inhibition of mitosis in the intestinal epithelium were maximal on days 1-2 and were most severe in the ileum, but mucosal lesions were recovered by 5-7 days. Crypt and villus cell populations were reduced most in the ileum (60-70%), followed by the jejunum (45-60%), and the duodenum (35-40%). Stomach and colon had few mucosal lesions. GI tissues assayed for platinum concentrations indicated no preferential localization of cisplatin in any segment of small intestine. Histologic evidence suggested that proliferating epithelial cells in the crypt are the major targets for cisplatin cytotoxicity.

Currently experiments are underway to determine if orally administered platinum-binding compounds (i.e., $\text{Na}_2\text{Ca-EDTA}$) can ameliorate the intestinal toxicity of cisplatin.

C. Natural Killer Lymphocytes

Recent results from our laboratory have demonstrated that tumor promoting phorbol diesters (PRDE) can interrupt host natural killer (NK) immunologic containment of tumors through at least three independent mechanisms: 1) PRDE's can inhibit host effector cell activity by inducing reactive oxygen formation by monocytes and polymorphonuclear leukocytes. 2) The ability of interferon to augment NK activity is both inhibited and abrogated by PRDE's. 3) Tumor cells ordinarily susceptible to NK lysis can be induced to become resistant by prolonged (24 hrs) exposure to PRDE's. This event is accompanied by induction of myeloid maturation in K562, apparently dependent on reactive oxygen generation by K562, and occurs at lower concentrations of TPA (20-50 pM) than any previously described effect of tumor promoters.

D. Mechanism of Action of Tumor Promoting Compounds

We have also demonstrated the presence of two specific receptors for ^3H -12-o-tetradecanoyl-phorbol-13-acetate (^3H -TPA) in human myeloid cells, only one of which can be found in lymphoid cells. Normal, mature cells do not differ from their immature precursors or transformed counterparts in number or affinity of ^3H -TPA receptors. The effects of PRDE's on human hematopoietic cells appear to be mediated by these specific receptors. We are further characterizing the nature and mechanism of action of specific receptors for tumor promoting PRDE's.

In collaboration with Dr. N. Colburn we have determined that the ability of mouse epidermal cells to undergo tumor promotion in response to PRDE's is related to alterations occurring after the ^3H -TPA receptor binding event, rather than to changes in number, binding affinity or down modulation of this receptor. While the mitogenic effect of TPA on these cells depends upon epidermal growth factor receptors, the determinants of promotability

by TPA have not yet been identified. Cell fusion experiments to date have indicated that promotability is a dominant trait in these cells and that all members of the present battery of variant cell lines fall into only one complementation group.

TPA-induced reactive oxygen generation appears to play an important role in tumor promotion since H_2O_2 alone can replace TPA as a promotor, and catalase can block the promoting effect of TPA.

E. Human Cancer-Identification of Critical Loci

The strategy chosen to identify human cancer-critical genetic loci consists of evaluation of fibroblasts from inherited cancer syndrome patients for expression of the transformed phenotype in culture. Studies with human fibroblasts have shown that density dependent inhibition of proliferation can be analyzed rapidly in an assay developed this year. Dermal fibroblasts from individuals at high risk for cancer due to the Basal Cell Nevus Syndrome show less density dependent inhibition of proliferation than normal controls. No difference in sensitivity to X-ray or UV irradiation could be demonstrated.

Dermal fibroblasts from patients with this gene defect did not differ from controls in response to PRDE's or in metabolism of antipromoting retinoids. Neither showed growth in soft agar in response to TPA, while both converted a fraction of retinol to retinoic acid, which is a much more active anti-promoting compound.

F. Epidermal Cell Resistance to Mitogenic Activity of TPA

This project focuses on the role of cell membrane transport of glucose in malignant transformation and in preneoplastic progression. The experimental system consists of stable mouse epidermal cell lines, well-characterized for their response, or lack of response, to tumor promoters and mitogens.

Preliminary results suggest that glucose transport may be a rate-limiting factor for the 12-o-tetradecanoylphorbol-13-acetate (TPA) mitogenic response, but not for the promotion response to TPA. There is a correlation between lower growth rate, low 2-deoxy-glucose (2DG) uptake, mitogenic responsiveness and a TPA-induced increase in 2DG uptake. These findings, using the mouse epidermal cell system, support evidence obtained from other cell systems that chemically and virally transformed cells take up 2DG at a higher rate than their non-transformed counterparts. Correlations with other membrane characteristics (both ultrastructural and physiologic) are also being investigated.

Significance to Biomedical Research and the Program of the Institute:

As stated in the objectives it is our intent to integrate pharmacological and morphological data to develop a better understanding of the mechanisms of toxicity of oncolytic agents. The importance of using morphological approaches

to study toxicology would be difficult to overstate. It is often the descriptive data that helps to narrow the range of other types of investigations into certain phenomena. Out of these studies should come new information that will improve our ability to chemotherapeutically treat cancer with minimal toxicity.

The development of a rapid, inexpensive cardiac myocyte model to assess the cardiotoxic potential of new oncolytic agents makes it possible to make programmatic decisions in the drug development area much more expeditiously than in the past.

The identification and quantification of receptor binding sites for tumor promoting agents will make it possible to better understand the mechanism(s) of tumor promotion by TPA.

Proposed Course:

1. The experiments with cisplatin are being phased-out due to changes in personnel.
2. The tumor promoting experiments with TPA will most likely be continued in another Division of the NCI better equipped for such important but hazardous studies.
3. Studies are currently underway to determine the mechanism by which the cardiotoxic anthracyclines adversely affect isolated rat cardiac myocytes.

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ANNUAL REPORT OF THE LABORATORY OF MEDICINAL CHEMISTRY AND BIOLOGY

DEVELOPMENTAL THERAPEUTICS PROGRAM

DIVISION OF CANCER TREATMENT

October 1, 1980 - September 30, 1981

The Laboratory of Medicinal Chemistry and Biology is an organizational entity resulting from the consolidation in 1975 of previously established sections from within the Drug Development and Drug Evaluation Branches of the Drug Research and Development Program, DCT; the Laboratory of Chemical Pharmacology, Experimental Therapeutics Program, DCT; and the Laboratory of Biochemistry, DCBD. The aim in establishing the new Laboratory was to bring about a closer relationship between medicinal chemists involved in the design and synthesis of new antitumor agents, and biologists involved in the elucidation of their biochemical pharmacology and in their clinical evaluation. It was felt in proposing this new organizational structure that knowledge of the molecular biology and etiology of cancer is approaching the level at which the biologist can now make useful early contributions to the rational design of new antitumor agents, rather than being restricted almost entirely to biochemical, pharmacological and clinical studies on agents whose antineoplastic activity has already been established on an empirical basis. While it is recognized that it would be premature and over-optimistic to propose that empirically-based screening approaches can in the foreseeable future be abandoned in favor of specific, rational antitumor drug design, the time now appears to have arrived when a mixture of empirical and rational approaches is warranted. Examples of drug classes in which opportunities for specific, target-directed design are now possible are agents affecting structure and function of tumor cell surface glycoproteins; inhibitors of viral oncogenesis; agents with specific or unique DNA-binding properties; transition-state inhibitors for key enzyme sites; and agents with specific tissue-localizing properties. In these and a number of other rapidly expanding research areas, the state of the art appears sufficiently advanced that the biologist with specific expertise can make contributions of value to the medicinal chemist in the design of new antitumor agents and in the modification of existing agents.

Conversely, from the point of view of the medicinal chemist, a closer inter-relationship with the molecular biologist and the clinician offers the opportunity of obtaining specific biological assay data with the minimum of delay, in order to guide the course of further synthesis. In addition to providing routine antitumor screening, the biologist can design assay methods tailored to the specific target site under consideration. Assays for parameters such as inhibition of viral replication, immunostimulation, enzyme inhibition or tumor cell membrane binding can be provided where relevant to the activity under consideration. Thus, the juxtaposition within a single laboratory of medicinal chemists and biologists from a variety of different disciplines offers the prospect of accelerating drug development, and of supplementing the traditional empirical approaches which have yielded most of our clinically successful antitumor drugs up to the present time with specific target-site directed drug design programs.

The major organizational entities within the original Laboratory of Medicinal Chemistry and Biology, in addition to the Drug Design and Chemistry Section, were the Molecular Biology and Methods Development Section, with the capacity to assess the effects of new agents on cell growth and cell cycle progression, and including a membrane biology group, with a specific interest in the role of tumor cell membrane alterations in drug resistance; and an Applied Pharmacology Section which is concerned both with the mode of action of new agents at the molecular level and with disposition and metabolic studies of such agents in experimental animals and at the Phase I level. The Office of the Chief, LMCB, in addition to assuming administrative responsibilities for the Laboratory as a whole, also maintains an active research interest in antitumor drug disposition studies, in the mode of action of new agents, and in the identification of potential sites for pharmacological attack. On March 1, 1980, the Laboratory underwent a major expansion, with the addition of two additional organizational components, i.e., the Biochemistry and Drug Interactions Sections. The Biochemistry Section is concerned with the mechanisms by which antitumor agents, and particularly the oncolytic analogues of the dicarboxylic amino acids exert their inhibitory effects on pyrimidine and purine biosynthetic pathways; while the Drug Interactions Section studies the effects on the action of antitumor drugs of factors such as metabolism, absorption, tissue distribution, protein binding and excretion. Throughout the Laboratory, particular stress is given to studies of agents originating from within the LMCB drug synthesis program.

As indicated above, this Laboratory has an interest both in new antitumor agents originating from the scientific community as a whole, and also in agents originating within our own Drug Design and Chemistry Section. Of the latter group, the compound AZQ (NSC-182986) is presently in Phase II clinical trial, spirohydantoin mustard (NSC-172112) is undergoing toxicology evaluation, and a Phase I clinical trial will likely be initiated with dihydro-5-azacytidine (NSC-264880) during the current fiscal year.

The HPLC assay for AZQ (NSC-172112) described in last year's report was used to measure the drug in the plasma, urine and CSF of 20 patients receiving dose-levels ranging from 1 to 20 mg/m² during a Phase I trial conducted in the Medicine Branch, DCT. All subjects showed a very rapid redistribution phase ($t_{1/2}(\alpha) = 2.8 \pm 1.3$ min) followed by a slower yet still fast plasma elimination phase. The mean elimination half-life for the terminal phase of the two-compartment open model to which the data was fitted was 33.3 ± 4.5 min and was dose independent. The pharmacokinetics in man closely paralleled those in the rat, dog and rhesus monkey. A mean total body clearance of 517 ± 155 ml/min implied the involvement of hepatic as well as renal clearance. The apparent volume of distribution at steady state averaged 15.8 ± 4.0 liters, suggesting a distribution only slightly less than the total extracellular water compartment.

The urinary excretion of unchanged AZQ was examined in 5 patients receiving 10 mg/m² or more of the drug. Measurable amounts of intact drug could be found in only two of these patients and represented less than 0.2% of the total administered dose. Cerebrospinal fluid, obtained via lumbar puncture was available from 3 different patients, including one from two different dosage cycles. AZQ was found to enter the CSF, and to attain concentrations that were substantial compared to plasma levels. Detailed studies of AZQ metabolism and pharmacokinetics will continue during Phase II clinical trials of the drug.

Considerable progress has been made in efforts to prepare cytidine deaminase inhibitors for potential use in combination with antitumor agents such as ara C whose clinical utility is limited by enzyme-catalyzed deamination in vivo. In the ring-expanded pyrimidine nucleoside series, three compounds have been prepared with K_i values in the range 1 to 5×10^{-8} M, i.e., approximately one order of magnitude more active than the reference compound tetrahydrouridine; the latter agent was the most active cytidine deaminase inhibitor described prior to the present studies, and the only compound of this type which has entered clinical trial. Biological evaluation of these compounds is continuing.

Efforts have continued in the design and synthesis of amino acid analogues which are activated to cytotoxic compounds by the enzyme tyrosinase, and which could therefore be anticipated to show activity against melanotic melanoma, a tumor with high levels of the latter enzyme. Of the three initial target compounds, two are readily oxidized substrates for the enzyme, and also exhibit inhibitory activity against melanotic B16 melanoma in vitro, with ID_{50} -values of 4.5×10^{-6} M and 7.5×10^{-6} M.

In another approach to the treatment of melanoma, syntheses of a number of catecholamine analogues of dopamine have been carried out. Recent studies in this laboratory have established that (a) the catechol hydroxyl groups of dopamine were required for antitumor activity and (b) the aminoethyl side chain of the molecule could be replaced with methyl or aminomethyl groups without abolition of antitumor activity. Oxidation of the catechol to an o-quinone which then reacted with sulfhydryl containing enzymes was suggested as a possible mechanism of action for this class of compounds. These considerations have led to the synthesis of a series of hydroxylated benzylamines, the antitumor activity of which is currently under evaluation.

A long-term interest of this Laboratory is the development of quantitative structure:activity correlations in the antitumor drug area, as an aid to antitumor drug design. An extensive study of the effect of structural variations on antitumor activity and toxicity within the colchicine series has recently been completed. Quantitative structure-activity studies indicated that there was no value in the continued modification of colchicine in the 7- and/or 10-positions since the equations obtained correlating structure with antitumor activity or toxicity were very similar. These relationships were not obeyed for 4-substitution, however, and the 4-formyl derivative has proven to be both more potent and less toxic than the parent compound in the P388 system in vivo. In view of these observations, 4-formylcolchicine has been prepared in large quantity, and submitted for more extensive evaluation in the complete tumor panel.

The development of new analytical techniques for agents of interest to LMCB has continued. The objectives of these studies are to establish the structure and purity of new antitumor agents, and also to elucidate reaction mechanisms and to develop assays for the quantitation of these agents in physiological samples. Mass spectrometry, gas-liquid chromatography and HPLC techniques are used. Compounds of current interest are aziridinylbenzoquinones, reduced pyrimidine nucleosides, seven-member ring nitrogen heterocycles, nitrogen mustards and cytidine deaminase inhibitors. The acid and base-catalyzed rearrangements of pyrimidine nucleosides are under investigation.

At the molecular level, studies have continued on the effects of nucleoside analogues on RNA synthesis per se, on the methylation of RNA, and also on the modifications of the translational activity of mRNA following the incorporation of these molecules; such studies appear to be a sensitive method for defining and assessing differences among the modes of action of these compounds as antitumor agents in vivo. Compounds or combinations of compounds of this class studied during the past year are 5-fluorouracil (5-FU), 5-FU plus PALA, 5-azacytidine, 5,6-dihydro-5-azacytidine, sangivamycin, thiosangivamycin and cordycepin (3'-deoxyadenosine). The following effects were observed.

1. The biological effects of N-(phosphonacetyl)-L-aspartate (PALA) and 5-FU were examined singly, and in combination on the growth of a human mammary carcinoma (MDA) cell line in culture. All combinations of 5-FU (2.5×10^{-7} to 1.5×10^{-5} M) and PALA (6.0×10^{-5} to 3.6×10^{-3} M) resulted in synergistic inhibition of cell growth as revealed by a 50% response isobologram. To examine the biochemical basis for the synergism, measurements of the incorporation of [^{3} H]5-FU into total non-poly(A)- and poly(A)- RNA, and simultaneous incorporation of [^{14} C]deoxyguanosine and [^{3} H]deoxyuridine into DNA were determined. The combination of 3.7×10^{-5} M PALA and 1×10^{-6} M 5-FU produced 65-85% inhibition of cell growth after continuous treatment for one to three days. Treatment of the cells for 3 or 24 hr with the same drug regimen produced approximately a 170% increase in the incorporation of 1×10^{-6} M [^{3} H]5-FU into poly(A)RNA in comparison to [^{3} H]5-FU treatment alone; exposure for 24 hr with 3.7×10^{-5} M PALA and 1×10^{-6} M [^{3} H]5-FU resulted in a 285% increase in the incorporation of [^{3} H]5-FU into non-poly(A)RNA. The incorporation of either [^{14} C]deoxyguanosine or [^{3} H]deoxyuridine into DNA was not inhibited by this drug regimen; however, the incorporation of [^{3} H]deoxyuridine into DNA was significantly elevated upon 12 and 24 hr of exposure to PALA alone. PALA and 5-FU treatment resulted in a 75% reduction in the concentration of UTP and no change in the concentration of 5-FUTP vs 5-FU treatment alone. Thus, the proportion of 5-FUTP in the total 5-FUTP + UTP pool was enhanced more than three-fold by the combination regimen. These results indicate that the synergistic effect of the combination of PALA and 5-FU on the growth of MDA cells correlates with an increased proportion of 5-FUTP in the pyrimidine nucleotide pool, and consequently with an enhanced incorporation of 5-FU into RNA, but not with inhibition of DNA synthesis.

2. The mechanism of action of adenosine, 2'-deoxyadenosine, and cordycepin (3'-deoxyadenosine) was explored in L1210 cells in vitro under conditions where their deamination was blocked by the adenosine deaminase inhibitor, dCF. Cordycepin but not adenosine or 2'-deoxyadenosine inhibited the methylation of nuclear RNA in the presence of dCF. Upon the addition of homocysteine, adenosine produced 65% inhibition of methylation of nuclear RNA, whereas 2'-deoxyadenosine was ineffective and the inhibitory effect of cordycepin was not potentiated. Under the latter conditions, RNA synthesis as measured by [^{14} C]-uridine incorporation was marginally affected (30% inhibition) by adenosine plus homocysteine, but markedly inhibited by 70% by cordycepin. Cordycepin inhibited 2'-O methylation of nuclear RNA to a greater extent than base methylation, while the combination of adenosine and homocysteine inhibited these sites to equal degrees. Moreover, cordycepin inhibited >18 S nuclear RNA four times as extensively as 4 S nuclear RNA, in comparison to the equal extents of inhibition of these two classes of nuclear RNA by adenosine plus homocysteine. A positive correlation was observed between the generation of intracellular S-adenosylhomocysteine in L1210 cells and the inhibition of methylation of

nuclear RNA by adenosine plus homocysteine, but not by cordycepin. These results indicate that a significant amount of S-adenosylhomocysteine can be generated in the presence of adenosine and homocysteine, presumably via S-adenosylhomocysteine hydrolase, leading to marked inhibition of methylation of nuclear RNA in mouse lymphoid leukemia cells.

3. The lethal and sublethal effects of sangivamycin were studied in sarcoma 180 *in vitro* in relation to drug concentration and duration of drug exposure. Sangivamycin lethality was found to be dependent on both drug concentration and duration of drug exposure. Pronounced effects on cell survival were observed only when sangivamycin exposure was prolonged; with prolonged drug exposure, small increments in sangivamycin concentration resulted in large increases in cell killing. Log phase cells were more susceptible to the lethal effects of sangivamycin than early plateau phase cells. Measurements of incorporation of [³H]thymidine and [³H]uridine into the acid-insoluble cell fraction demonstrated inhibition of both DNA and RNA synthesis by sangivamycin which was also dependent on drug concentration and duration of drug exposure, reflecting the lethality characteristics of sangivamycin. As sangivamycin concentration was increased, DNA synthesis was inhibited more rapidly than RNA synthesis. Flow cytometry demonstrated a concentration and time dependent accumulation of cells in the late S and G₂M region of the DNA histogram. Our findings indicate that maximum lethality is obtained by prolongation of sangivamycin exposure, and suggest that pharmacokinetic studies may be important for determining regimens which provide such exposure in man.

4. The pyrrolopyrimidine, sangivamycin, and the adenosine analog, xylosyladenine, were examined for their effects on the synthesis and methylation of polysomal RNA in Ehrlich ascites tumor cells *in vitro*. The synthesis of non-polyadenylic acid (poly(A))- and poly(A)-containing RNA was inhibited 50% at concentrations of 7×10^{-6} M and 3×10^{-6} M, xylosyladenine, respectively, when adenosine deaminase was inhibited with 2'-deoxycoformycin. Sangivamycin inhibited the synthesis of non-poly(A)- and poly(A)RNA by 50% at concentrations of 5×10^{-5} M and 2×10^{-5} M, respectively. Electrophoretic separation of non-poly(A)RNA into rRNA and tRNA indicated that the inhibitory effects of both drugs were more pronounced on 28S than on 18S rRNA, and that xylosyladenine but not sangivamycin inhibited the synthesis of tRNA. Assessment of the effects of both analogs on the methylation of polysomal RNA revealed that xylosyladenine inhibited the methylation of non-poly(A)- and poly(A)RNA while sangivamycin only weakly affected the latter species of RNA. Base methylation of the affected species of RNA was inhibited slightly more than 2'-O-methylation by both drugs. These results indicate that sangivamycin is a more selective inhibitor of polysomal RNA in comparison to xylosyladenine under conditions where adenosine deaminase is not a limiting factor.

5. In further studies of the effects of the adenosine deaminase inhibitor 2'-deoxycoformycin (dCF), levels of dCF in plasma, plasma concentrations of adenosine and deoxyadenosine and urine levels of deoxyadenosine were measured in 19 leukemic patients undergoing treatment with the drug at dose-levels ranging from 0.25 to 1.0 mg/kg. Plasma concentrations of adenosine and deoxyadenosine and the urine concentration of deoxyadenosine did not correlate with dCF dose, therapeutic response or toxicity. At the molecular level, the immunosuppressive effect of dCF is being studied *in vitro*, utilizing T and B lymphocyte cultures from mouse spleen and thymus. Processes currently being assessed are suppression of mitogen-induced blastogenesis, nucleic acid synthesis and

formation of adenosine metabolites such as dATP. Application of these findings to lymphoblasts from lymphocytic leukemia patients and to a human T-cell lymphoma cell line in culture is planned.

The Biochemistry Section, LMCB, examines the biochemical mechanisms underlying the therapeutic activity and the toxicity of new oncolytic drugs, and devises rational methods for increasing therapeutic activity and reversing toxicity. Recent studies have been concerned with the mode of action of the antitumor agents PALA (N-phosphonacetyl-L-aspartic acid), Acivicin (AT-125), L-alanosine, DON, and the Robins thiazole nucleoside NSC 286193D.

With reference to the latter compound, when an examination was made of the ability of a comprehensive series of preformed purines and pyrimidines to overcome the inhibition of thymidine incorporation provoked by exposure to the thiazole nucleoside, the guanines were notably effective, but xanthosine also was shown to be an active antidote. Confirmation that the drug was producing a state of guanine deprivation was provided by HPLC analogs of acid-soluble extracts of P388 cells grown in culture: a time-dependent fall in the concentration of GTP ensued upon exposure to 10 μ M thiazole; ATP was unaffected and UTP increased.

Pursuant to these findings, an examination was made of the enzymologic steps unique to guanine biosynthesis in cells exposed to cytotoxic concentrations of the drug. No inhibition of GMP synthetase (XMP aminase) could be demonstrated either in vivo or in vitro, but the specific activity of IMP dehydrogenase underwent substantial reductions in both of these cases. A preliminary kinetic analysis of this interaction revealed that the thiazole nucleoside inhibited a partially purified preparation of IMP dehydrogenase from P388 cells uncompetitively with IMP, and exhibited a K_i of ~ 100 μ M. Since concentrations equal to or greater than this value are reached and maintained in subcutaneous nodules of this tumor following therapeutic doses of the drug, it is possible that the unchanged drug plays a role in its activity. However, by analogy to other oncolytic nucleosides, it is additionally likely that anabolism to the nucleotide occurs and that this molecule participates in the mechanism of action of the drug.

With reference to the L-glutamine antagonist DON (6-diazo-5-oxo-L-norleucine, NSC-7365), interest in this compound was renewed as a consequence of the finding of its effectiveness in treating human lung and colon xenografts in athymic mice. We have used the native DON-sensitive P388 leukemia as a model to study the mechanism of action of DON. [$6\text{-}^{14}\text{C}$]DON was transported by P388 cells with a K_m of 0.66 mM and a V_{max} of 0.33 nmoles of the drug transported/million cells/min, i.e., at a rate about 20 times slower than L-glutamine. L-Glutamine competitively inhibited this transport. Four hours following the intraperitoneal administration of DON (20 mg/kg) to tumor bearing mice, PRPP amidotransferase, GMP synthetase, fructose-6-phosphate amidotransferase, L-asparagine synthetase and L-glutaminase activities were potently inhibited (>50%), whereas the specific activity of carbamyl-phosphate synthetase was only marginally repressed; dialysis against buffer of the extracts prepared from treated tumors failed to reverse inhibition of the enzyme activities. Maximum inhibition of susceptible L-glutamine amidotransferases was observed within 4-8 hours of the administration of DON; complete restitution of the enzyme activities was achieved in 48 hours. Analysis of acid-soluble nucleotide pools after treatment with DON

indicated a reduction in the concentration both of adenine and guanine nucleotides. Furthermore, the synthesis of RNA and DNA by cells in culture was potently inhibited, whereas protein synthesis was not affected. A study of the ability of purines, pyrimidines and amino acids to reverse the cytotoxicity of DON to P388 cells revealed that adenine, adenosine, hypoxanthine and L-glutamine were effective counteragents. These studies confirm that DON behaves principally as an inhibitor of the de novo purine biosynthetic pathway.

In view of the limited clinical activity of the aspartate transcarbamylase (ATCase) inhibitor PALA (N-(phosphonacetyl)-L-aspartic acid; NSC-224131), in contrast to its wide range of activity in experimental tumor systems, studies have continued to elucidate mechanisms of natural and acquired resistance to this agent. A variant of the Lewis lung Carcinoma (LL) resistant to PALA was developed by exposure to sub-therapeutic doses of the drug. This variant showed markedly elevated activities of the first 3 enzymes of pyrimidine biosynthesis [e.g. carbamyl phosphate synthetase II (CPS II) + 3x]. Inasmuch as mammalian CPS II is subject to endogenous regulation, measurements were made of these effectors in the PALA-sensitive and resistant tumors. The net concentrations of uridine nucleotides (Σ U) (500 μ M) and of PRPP (55 μ M) were elevated 2-fold in the PALA-resistant variant when compared to the parent line (285 and 29 μ M). Since these intermediary metabolites exert opposite effects on CPS II (PRPP(+), Σ U(-)), the unidirectional changes in them probably tend to cancel out, leaving the enzyme functioning normally. To determine the enzymologic basis for the elevated levels of these effectors, the systems principally responsible for the generation and consumption of UTP and PRPP were measured. Enhanced de novo pyrimidine biosynthetic activity in the resistant variant may explain the augmented pool of Σ U. Treatment of mice bearing sensitive or resistant LL with PALA (400 mg/kg) provoked sharp decreases (70-80%) in Σ U and PRPP. The decrease in Σ U is due to inhibition of the de novo pathway. PALA also provokes elevations in purine nucleotide pools (50-100%) which may reflect increased utilization of PRPP and thus diminished levels. However, PRPP synthetase, PRPP amidotransferase, orotate phosphoribosyl transferase, PRPP phosphohydrolase and HGPRTase were present at equivalent specific activities in both lines.

Studies have continued on the mechanism of resistance to the clinically useful alkylating agent melphalan. The availability of the melphalan sensitive and resistant lines of L1210 cells in primary culture permitted an analysis of their sensitivity to a variety of agents which could serve as biochemical probes. Growth of cell types were equally inhibited by a wide variety of cytotoxic agents including microtubule and microfilament poisons. Both cell types were equally sensitive to the sulfhydryl reagents iodoacetamide and N-ethylmaleimide. However, the melphalan resistant line was also 3 to 5 times resistant to the poorly permeable mercurial p-chloromercuribenzenesulfonate (PCMBs). This differential cytotoxicity was also established by clonal growth and is accompanied by a major swelling and lysis of the cells. The initial binding of [^{203}Hg]PCMBs was the same for melphalan sensitive and resistant cells, and no increase in release of label was seen in the resistant cells upon incubation in mercury-free medium. Treatment of cells with 5 μ M PCMBs resulted in 4 times as much uptake of $^{22}\text{Na}^+$ by the sensitive cells. At 50 μ M PCMBs, three times as much $^{45}\text{Ca}^{++}$ was taken up by the sensitive cells. However, $^{42}\text{K}^+$ release was not an indicator of PCMBs differential activity in the melphalan sensitive and resistant lines. When both cell lines were exposed to mechlorethamine at 25 μ M for 30 min, five times as much $^{45}\text{Ca}^{++}$ was taken up

by the sensitive L1210 cells. These differential responses to PCMBs and alkylating agents as measured by survival and membrane permeability to ions may be related to the biochemical basis of resistance.

A major concern of the Drug Interactions Section has been the dose-limiting pulmonary toxicity of bleomycin. It has been proposed that the interaction of bleomycin with Fe^{2+} forms a potent redox cycling system. The Drug Interactions Section has studied the interaction of bleomycin A₂ with pulmonary microsomes to determine if this interaction results in deoxyribose cleavage. Unlike mitomycin C, the incubation of bleomycin with rat lung microsomes, in the presence of NADPH and O_2 , does not result in lipid peroxidation. However, if DNA is present significant cleavage of the deoxyribose moiety results. This bleomycin-mediated DNA cleavage is NADPH-dependent (although NADH can also serve as an electron donor), oxygen-dependent and dependent on the type of nucleic acid employed. Furthermore, it was observed that incubation of bleomycin with microsomes in the absence of DNA resulted in the inactivation of bleomycin upon subsequent addition of DNA. This inactivation of bleomycin by lung microsomes is also oxygen and NADPH dependent. Preliminary studies have demonstrated that purified NADPH cytochrome P-450 reductase can also mediate bleomycin-DNA chain breakage.

In further study of this effect, we have attempted to determine whether reactive oxygen contributes to bleomycin-mediated DNA cleavage following interaction with rat lung microsomes by two approaches: (1) by examining the effects of reactive oxygen scavengers and (2) by examining the effects of redox cycling agents on this reaction. It was observed that superoxide dismutase and dimethylurea, a hydroxyl radical scavenger, significantly inhibited bleomycin-mediated DNA cleavage implicating the involvement of reactive oxygen. This reaction was also inhibited by cytochrome c, NBT, EDTA and glutathione. On the other hand, ascorbic acid significantly enhanced this reaction. The addition of the redox cycling agents mitomycin C, nitrofurantoin or paraquat also significantly enhanced the reaction. The enhancing effect of these redox cycling agents was significantly inhibited by superoxide dismutase. Furthermore, bleomycin significantly inhibited the stimulation of lipid peroxidation by these carriers implying that reactive oxygen was diverted to the bleomycin-DNA complex rather than to microsomal phospholipids. This study confirms that reactive oxygen is involved in bleomycin-mediated DNA cleavage by a biological system.

We have recently observed that the interaction of bleomycin with lung microsomes also results in chemiluminescence. The time course is similar to the time course for both bleomycin-mediated DNA cleavage and bleomycin inactivation following the interaction of bleomycin with microsomes. Preliminary studies have indicated that the addition of paraquat to the microsomal system not only enhances bleomycin-mediated DNA cleavage but also stimulates bleomycin-inactivation and bleomycin-chemiluminescence. From these studies we propose that following the interaction of bleomycin with either chemical or biological free radical generating systems some portion of the bleomycin molecule is activated to an electronically excited state which can pass this excess energy to DNA, resulting in deoxyribose cleavage, or in the absence of DNA decomposes to an inactivated state. Studies are currently in progress to develop this model as to better understand the molecular mechanisms involved in bleomycin's chemotherapeutic activity and also cytotoxic activity against lung cells.

Within the Office of the Chief, studies have continued on agents which affect microtubule structure and function. Taxol, a unique antimitotic agent which seems to act by provoking the uncontrolled polymerization of tubulin, was found to have highly variable effects on both tubulin polymerization and GTP hydrolysis. The typical microtubule assembly reaction has three essential components besides tubulin: microtubule-associated proteins, GTP and a warmer temperature. If taxol was added, any one of the other components could be eliminated and tubulin would polymerize. Thus at 0°, polymerization would occur with microtubule-associated proteins and GTP, while at 37° polymerization would occur with either microtubule-associated proteins or GTP. When GTP was required with taxol, GTP hydrolysis and polymerization occurred simultaneously at 1:1 stoichiometry. When GTP was not required, hydrolysis occurred independently of polymerization.

Antitumor agents which attack tubulin and prevent microtubule formation have unexpected effects on the GTPase activity of tubulin. Not unexpectedly, vinblastine and maytansine, competitive inhibitors in binding to tubulin, potently inhibit the hydrolysis of GTP. Colchicine and nocodazole, two drugs that bind competitively at a different site on tubulin, potently stimulate the GTPase reaction. Finally, however, podophyllotoxin, which competes with colchicine and not vinblastine in binding to tubulin, inhibits GTP hydrolysis. This somewhat paradoxical result apparently derives from a distinct structural feature of podophyllotoxin (a tetrahydronaphthol moiety) which is not present in colchicine and nocodazole. Trimethoxybenzene structures, which represent the overlapping structural analogies in colchicine, nocodazole and podophyllotoxin, do stimulate GTP hydrolysis by tubulin.

DeoxyGTP analogs were equal or superior to GTP in supporting the polymerization reaction, while analogs bearing substituents on the 2'- or 3'-hydroxyls were markedly inferior to GTP. The substituted GTP analogs also were unable to inhibit GTP-supported polymerization. These findings demonstrate that steric factors involving the ribose moiety of GTP reduce the affinity of a nucleotide for the exchangeable site. Since other workers have found that a bulky substituent on the guanine moiety does not affect nucleotide binding at the exchangeable site, this implies that the ribose moiety is located at the interior of the binding site.

GDP is a potent inhibitor of tubulin polymerization, but no analog of GDP was as effective as GDP itself in inhibiting polymerization. This included the deoxyGDP analogs, even though the deoxyGTP analogs were effective substrates. Most notably 2',3'-dideoxyGTP had no detectable inhibitory activity. In fact, the order of activity of GTP and its analogs in supporting tubulin polymerization bore no relationship to the order of inhibitory activity of GDP and its analogs in inhibiting the reaction. This implies fundamental differences in the mechanism of binding of GDP and GTP to tubulin and led us to suggest that there might be two mutually exclusive exchangeable nucleotide binding sites on tubulin. One site has a high affinity for GTP and when it is occupied tubulin is polymerizable. The other site has a high affinity for GDP and when it is occupied tubulin is non-polymerizable.

The preceding outline summarizes the objectives of the Laboratory of Medicinal Chemistry and Biology, and describes some of the research carried out within the Laboratory during the year. The bibliography for the Laboratory as a whole is listed below, followed by the individual Project Reports which describe this research in greater detail.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07102-06 LMCB																				
PERIOD COVERED October 1, 1980 to September 30, 1981																						
TITLE OF PROJECT (80 characters or less) Tubulin Structure and Microtubule Formation as Sites for Pharmacological Attack																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Ernest Hamel, M.D., Ph.D.</td> <td style="width: 20%;">NCI Cancer Expert</td> <td style="width: 10%;">LMCB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>Chii Mei Kuo Lin</td> <td>Biologist</td> <td>LMCB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Michael C. Lowe, Ph.D.</td> <td>NCI Cancer Expert</td> <td>LCP</td> <td>NCI</td> </tr> <tr> <td></td> <td>Anthony A. del Campo</td> <td>Biologist</td> <td>LCP</td> <td>NCI</td> </tr> </table>			PI:	Ernest Hamel, M.D., Ph.D.	NCI Cancer Expert	LMCB	NCI	Other:	Chii Mei Kuo Lin	Biologist	LMCB	NCI		Michael C. Lowe, Ph.D.	NCI Cancer Expert	LCP	NCI		Anthony A. del Campo	Biologist	LCP	NCI
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SUMMARY OF WORK (200 words or less - underline keywords) The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interactions between the polypeptide <u>subunits</u> of tubulin and its two tightly bound guanine nucleotides. Interactions of ribose-modified <u>GDP</u> and <u>GTP</u> analogs with tubulin were examined and suggested that there are actually two mutually exclusive exchangeable nucleotide binding sites. <u>Antimitotic drugs</u> were examined for their effects on tubulin-dependent <u>GTP hydrolysis</u>. The stimulatory effect of <u>colchicine</u> appears to derive from its <u>trimethoxybenzene moiety</u>. <u>Nocodazole</u> also stimulated <u>GTP hydrolysis</u>, while <u>maytansine</u> inhibited the reaction. Requirements for <u>GTP</u> and its hydrolysis were profoundly affected by other reaction components in the presence of <u>taxol</u>. <u>Hydrophobic chromatography</u> was successful in separating <u>α-tubulin</u> from <u>β-tubulin</u>.																						

Objectives:

Microtubules are reversibly formed protein structures present in all eucaryotic cells. One of their most important functions is participation in cell division, as they form the framework of the mitotic spindle. Microtubule structure and function are sensitive to a number of antineoplastic drugs, including colchicine, the clinically established vinca alkaloids, and two drug classes presently undergoing clinical trials, the podophyllotoxins and the maytansinoids. It is the goal of this project to assist in the development of effective new anti-cancer agents interfering with microtubule function.

The major constituent of microtubules is an acidic protein known as tubulin, which consists of two different polypeptide chains. Tubulin tightly binds two mols of GTP per mol of protein. Although distinct, the tubulin subunits are highly similar in molecular weight, amino acid composition, and isoelectric point and have never been separated unless chemically modified.

One mol of GTP is nonexchangeable with free GTP, cannot be removed from tubulin without denaturing the protein, and is unaltered in microtubule assembly and disassembly. Its function is entirely unknown. The other mol of GTP is freely exchangeable with unbound GTP or GDP and can be removed from tubulin by treatment with charcoal or alkaline phosphatase. This exchangeable GTP is hydrolyzed in microtubule assembly.

The formation of the tubulin dimer from its subunits is an obvious potential locus for the action of antineoplastic agents. The rational development of such drugs would be greatly aided by a greater understanding of the interactions between the polypeptide chains of tubulin and the two bound GTP molecules. It is therefore a major goal of this project to develop a method of separating the two subunits of tubulin and then to reconstitute active protein from the separated subunits and guanine nucleotides.

The intimate involvement of GTP in the structure of tubulin and in the formation of microtubules suggests that guanine and guanosine analogues may interfere with microtubule function. A second major goal of this project is to explore this possibility.

Major Findings and Proposed Course:

1. We have continued our studies on interactions of GDP and GTP analogs at the exchangeable nucleotide binding site of tubulin by examining analog effects on tubulin polymerization. Initial studies have been performed with ribose-modified analogs.

DeoxyGTP analogs were equal or superior to GTP in supporting the polymerization reaction, while analogs bearing substituents on the 2'- or 3'-hydroxyls were markedly inferior to GTP. The substituted GTP analogs also were unable to inhibit GTP-supported polymerization. These findings demonstrate that steric factors involving the ribose moiety of GTP reduce the affinity of a nucleotide for the exchangeable site. Since other workers have found that a bulky substituent on the guanine moiety does not affect nucleotide binding at the exchangeable site, this implies that the ribose moiety is located at the interior of the binding site.

GDP is a potent inhibitor of tubulin polymerization, but no analog of GDP was as effective as GDP itself in inhibiting polymerization. This included the deoxyGDP analogs, even though the deoxyGTP analogs were effective substrates. Most notably 2',3'-dideoxyGTP had no detectable inhibitory activity. In fact, the order of activity of GTP and its analogs in supporting tubulin polymerization bore no relationship to the order of inhibitory activity of GDP and its analogs in inhibiting the reaction. This implies fundamental differences in the mechanism of binding of GDP and GTP to tubulin and led us to suggest that there might be two mutually exclusive exchangeable nucleotide binding sites on tubulin. One site has a high affinity for GTP and when it is occupied tubulin is polymerizable. The other site has a high affinity for GDP and when it is occupied tubulin is non-polymerizable.

2. We have continued our studies on the effects of organic acids on the stabilization of tubulin and the induction of tubulin polymerization. We have extended our studies to associated GTP hydrolysis and performed electron microscopic examination of the polymer formed.

One significant finding was that two other anions 2-(N-morpholino)ethanesulfonate (Mes) and piperazine-N,N'-bis(2-ethanesulfonate) (Pipes), which are used almost ubiquitously by other workers in studying tubulin, are by themselves able to induce purified tubulin to polymerize at higher anion concentrations provided GTP is present. Interestingly, Mes and Pipes had little stabilizing effect on tubulin.

Polymerization was also induced by a number of potent stabilizing anions: glutamate, glutarate, fructose-1,6-(bis)phosphate, glucose-1-phosphate and creatine phosphate. On electron microscopic examination we observed microtubules with Mes as well as with heat-treated microtubule-associated proteins (which have no GTPase activity); sheets of protofilaments with Pipes, glutamate, glutarate, fructose-1,6-(bis)phosphate and glucose-1-phosphate; and a meshwork of interconnecting rings with creatine phosphate. It is of interest that all the anions which induce sheet formation are bifunctional, except for glucose-1-phosphate.

Morphology, however, did not correlate with the GTP hydrolysis associated with polymerization. With both glutamate (sheets) and microtubule-associated proteins (tubules) GTP hydrolysis and polymerization occurred simultaneously at a 1:1 stoichiometry (GTP hydrolyzed:tubulin polymerized). With Mes (tubules), Pipes (sheets), glutarate (sheets), fructose-1,6-(bis)phosphate (sheets) and glucose-1-phosphate (sheets) hydrolysis began before polymerization, and the amount of GTP hydrolyzed was substantially greater than the amount of tubulin polymerized. With creatine phosphate (interconnecting rings) little GTP was hydrolyzed and, unlike the other reactions, nucleotide was not required for polymerization.

3. Some antimitotic drugs which attack tubulin and prevent microtubule formation have unexpected effects on the GTPase activity of tubulin. Not unexpectedly, vinblastine and maytansine, competitive inhibitors in binding to tubulin, potentially inhibit the hydrolysis of GTP. Colchicine and nocodazole, two drugs that bind competitively at a different site on tubulin, potentially

stimulate the GTPase reaction. Finally, however, podophyllotoxin, which competes with colchicine and not vinblastine in binding to tubulin, inhibits GTP hydrolysis. This somewhat paradoxical result apparently derives from a distinct structural feature of podophyllotoxin (a tetrahydronaphthol moiety) which is not present in colchicine and nocodazole. Trimethoxybenzene structures, which represent the overlapping structural analogies in colchicine, nocodazole and podophyllotoxin, do stimulate GTP hydrolysis by tubulin.

Taxol, a unique antimitotic agent which seems to act by provoking the uncontrolled polymerization of tubulin, was found to have highly variable effects on both tubulin polymerization and GTP hydrolysis. The typical microtubule assembly reaction has three essential components besides tubulin: microtubule-associated proteins, GTP and a warmer temperature. If taxol was added, any one of the other components could be eliminated and tubulin would polymerize. Thus at 0°, polymerization would occur with microtubule-associated proteins and GTP, while at 37° polymerization would occur with either microtubule-associated proteins or GTP. When GTP was required with taxol, GTP hydrolysis and polymerization occurred simultaneously at 1:1 stoichiometry. When GTP was not required, hydrolysis occurred independently of polymerization.

4. We have succeeded in separating the α and β subunits of tubulin by hydrophobic chromatography, although the separation is not yet perfected.

Our future plans include refinement of the separation of the tubulin subunits; structural analysis of the subunits; and preparation of antibodies, including monoclonal antibodies, to the subunits, to assist in mapping of functional domains. We also can now undertake reconstitution of activity from the subunits, and we anticipate that the study we have previously made of tubulin stabilization will benefit our efforts. When conditions for reconstitution are discovered, we plan to undertake an analysis of the structural requirements for the nonexchangeable nucleotide. This may reveal information about its function and permit development of antitubulin agents. Finally, we are continuing our analysis of the structural requirements of the exchangeable nucleotide binding site. Guanine-modified analogs will be examined, as well as diphosphate and triphosphate derivatives of acyclovir [9-(2-hydroxyethoxymethyl)guanine], a guanosine analog lacking the 2' and 3' positions of the ribose ring.

Publications:

Hamel, E., Johnson, G., and Glaubiger, D.: Pharmacokinetics of leucovorin rescue using a new methotrexate-independent biochemical assay for leucovorin and N⁵-methyltetrahydrofolate. Cancer Treat. Rep. (in press), 1981.

Hamel, E. and Lin, C.M.: Glutamate-induced polymerization of tubulin: Characteristics of the reaction and application to the large-scale purification of tubulin. Arch. Biochem. Biophys. (in press), 1981.

Hamel, E. and Lin, C.M.: Interactions of tubulin with ribose-modified analogs of GTP and GDP: Evidence for two mutually exclusive exchangeable nucleotide binding sites. Proc. Natl. Acad. Sci. USA (in press), 1981.

Hamel, E. and Lin, C.M.: Stabilization of the colchicine binding activity of tubulin by organic acids. Biochim. Biophys. Acta (in press), 1981.

Lin, C.M., Hamel, E., and Wolpert-DeFilippes, M.K.: Binding of maytansine to tubulin: Competition with other mitotic inhibitors. Res. Commun. Chem. Pathol. Pharmacol. (in press), 1981.

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Drug Design and Chemistry Section
Laboratory of Medicinal Chemistry and Biology

October 1, 1980 - September 30, 1981

Scope

The objectives of the Drug Design and Chemistry Section of the Laboratory of Medicinal Chemistry and Biology are: (1) chemical research in the design and synthesis of new drugs for the treatment of cancer, (2) the chemistry of agents and drugs of interest in the treatment of cancer, (3) research on the structure, purity, decomposition products and metabolites of antitumor drugs by analytical instrumental techniques, (4) the quantitative and qualitative analysis of anticancer drugs in physiological fluids, (5) the development of structure-activity techniques to summarize existing data and to provide guidelines for analog synthesis and (6) the promotion of the interdisciplinary approach to the synthesis and evaluation of rationally designed drugs by means of collaboration between chemical and biological scientists.

Staff

The staff consisted of four senior scientists, one scientist, one NIH Visiting Scientist, one IPA, and one secretary.

Summary of Accomplishments

Approximately seven man-years of effort were devoted to the intramural research described in Individual Project Reports Z01-CM-03580, Z01-CM-03581 and Z01-CM-03582 during the period covered. Several compounds developed somewhat earlier in this laboratory progressed toward clinical trial. AZQ (NSC 182986) completed Phase I clinical trials and started Phase II trials at several institutions. Our research involvement continues with this drug through collaboration in the clinical trial at NIH and the BCRP. Spirohydantoin mustard (NSC 172112) has started toxicology evaluation prior to a Phase I study. Toxicology will be completed on dihydro-5-azacytidine (NSC 264880) during the current fiscal year.

Several excellent new inhibitors of cytidine deaminase were prepared in the ring expanded pyrimidine nucleoside series for combination studies with ara-C and other 4-aminopyrimidine nucleoside antitumor agents. One of these new compounds is the most effective known inhibitor of the human liver deaminase. Significant new chemistry was discovered when the role of mercury catalysis in the formation of O- and N-nucleosides from non-aromatic aglycones was investigated. The originally published structure of the Chinese plant product, squamalone, was determined to be in error and was corrected. A potential inhibitor of purine nucleoside transport was synthesized. Amino acid analogs which might be activated by tyrosinase to cytotoxic compounds were prepared and shown to be more active against melanotic than amelanotic B16 melanoma cells in vitro. Several trihydroxybenzylamines capable of being oxidized to ortho-quinones were prepared and found to have in vivo P388 leukemia activity.

Quantitative structure-activity studies indicated that there was no value in the continued modification of colchicine in the 7- and/or 10-positions since the equations obtained correlating structure with antitumor activity or toxicity were very similar. These relationships were not obeyed for 4-substitution, however, and the 4-formyl derivative has proven to be both more potent and less toxic than the parent compound. A method for deriving a tumored LD₅₀ value from screening toxicity day survivor data was devised. An excellent correlation in the colchicine series was obtained between the LD₅₀ values determined this way and LD₅₀ values obtained with untumored mice in the usual manner.

The analytical chemistry and quantitative analysis of AZQ in both animal and human biological samples was studied by HPLC techniques. The human pharmacokinetic behavior of AZQ was determined during Phase I trials in the NIH Clinical Center and Phase I/II trials at the BCRP in collaboration with clinicians at these hospitals. AZQ also was found to penetrate into the CSF in significant quantities. An acid catalyzed rearrangement, in which certain nucleoside ribofuranose derivatives were converted to ribopyranose compounds was discovered. This reaction was shown to convert tetrahydrouridine into a cytidine deaminase inhibitor with greatly reduced potency.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03580-12 LMCB																														
PERIOD COVERED October 1, 1980 to September 30, 1981																																
TITLE OF PROJECT (80 characters or less) Chemical Research in the Development of New Anticancer Drugs																																
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SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. The following topics are of current interest: (1) <u>pyrimidine nucleosides</u> as antitumor agents and <u>transition-state enzyme inhibitors</u>, especially inhibitors of <u>cytidine deaminase</u>, (2) natural product analogs of <u>ellagic acid</u>, <u>showdomycin</u> and <u>colchicine</u> and (3) <u>antimelanoma</u> agents based on <u>catechols</u>, <u>quinoneimines</u> and <u>quinomethides</u>.																																

Project Description:General Objective:

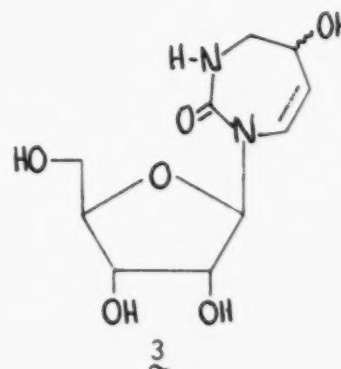
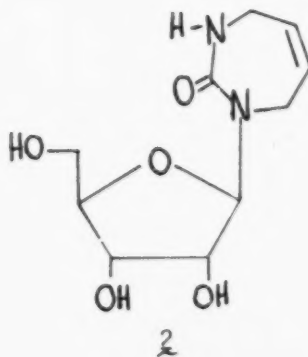
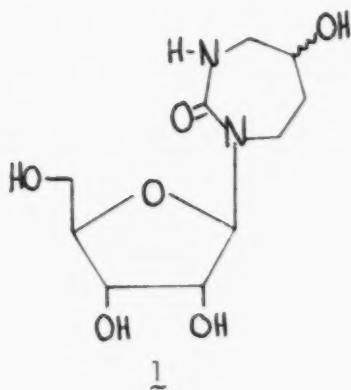
The objective of this project is the discovery of new types of drugs which are clinically useful against cancer. Medicinal chemical research is directed toward the synthesis of new compounds which have potential as useful agents. Leads for this program are generated from structure-activity studies, the DTP screening program, the literature, and biochemical rationale.

Specific Objectives:

1. The design and synthesis of transition-state inhibitors of the enzyme cytidine deaminase;
2. The preparation of analogs of natural products in order to overcome problems with the parent drugs;
3. The design and synthesis of agents specific for melanoma;
4. Exploitation of a new agent lead in the catechol family.

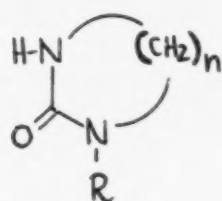
Major Findings:A. Nucleosides1. Transition-State Inhibitors of Cytidine Deaminase (Dr. Marquez):

a. Mechanism of Action and AraC Combination Studies. The mechanism of cytidine deaminase (CDA) inhibition of target compounds 1-3 has been studied in detail. All three compounds are reversible and competitive inhibitors of CDA. The activities are practically the same with K_i values ranging from 1 to 5×10^{-8} M. The most active compounds 1 and 2 were selected for further combination studies with AraC in CDA producing S-180 cells. These cell culture studies were performed by Dr. Vistica (LMCB). Compound 1 very effectively inhibited CDA from S-180 cells with a K_i (2×10^{-8} M) consistent with our previous results from both mouse kidney and human liver. Despite such activity, AraC + 1 and AraC + 2 combinations produced no change in the AraC dose dependent curve from 10^{-9} to 10^{-5} M. The concentration of 1 and 2 was held constant at 10^{-3} M. Similar negative results were obtained with the AraC + tetrahydrouridine combination.



Larger amounts of compound 2, the most synthetically accessible of the three, are being prepared for in vivo combination studies with AraC. In these studies it is hoped that if the host's enzyme plays an important role in the degradation of AraC, the combination should increase the cytotoxicity of AraC, especially at the lower dose levels. The syntheses and evaluation of all the regio- and diastereoisomers of 1 and 3 has been completed. All these materials showed less activity against CDA than 1, 2, and 3.

b. A Structure-Activity Relationship Study in Unsubstituted Cyclic Urea Nucleosides. These studies were aimed at determining the ideal ring size of the aglycon for maximum enzyme binding and inhibition of CDA. The optimum ring-size was seven member in both in the mouse kidney and human liver enzyme systems.



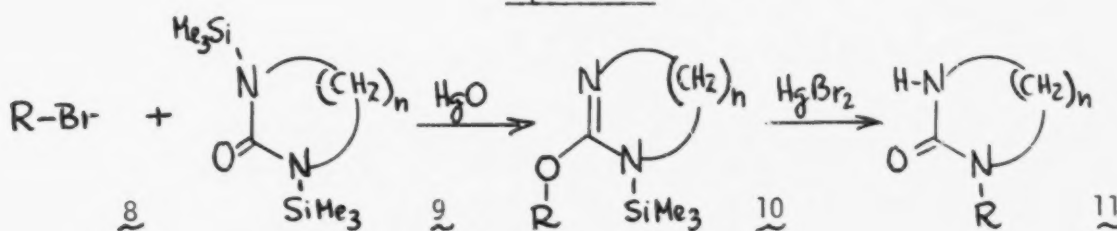
human liver, K_i (M)

<u>4</u> , $n = 2$	3×10^{-4}
<u>5</u> , $n = 3$	2.8×10^{-6}
<u>6</u> , $n = 4$	4×10^{-7}
<u>7</u> , $n = 5$	1.5×10^{-5}

R = β -D-ribofuranosyl

c. Nucleoside Synthesis. Mechanistic Study. The new condensation reaction originally discovered for the syntheses of compounds 1-3 was found to be of general use and applicability to all saturated cyclic ureas. A detailed study applied to the syntheses of compounds 4-7 revealed that all these reactions proceed through the intermediacy of the O-nucleosides (10). The O-nucleosides have been isolated, characterized, and rearranged quantitatively to the desired N-nucleosides (11). The specific action of each ingredient of the catalytic mixture employed was studied in detail and it is summarized in equation 1.

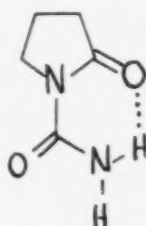
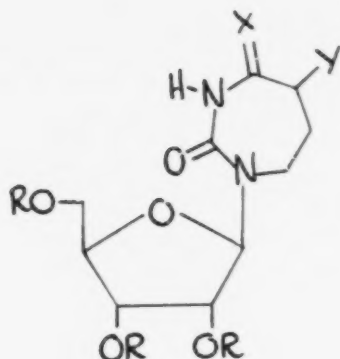
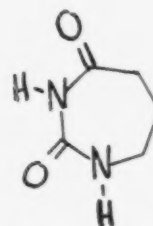
Equation 1



R = tri-O-benzoyl- β -D-ribofuranosyl

The complete understanding of this mechanism and knowledge of the exact structure of the persilylated ureas (identified as bis-N,N-trimethylsilyl derivatives by ^{13}C -NMR) led to the use of a new catalyst, trimethylsilyl triflate, which catalyzed the overall reaction starting with the persilylated urea and the simpler tri-O-benzoyl-1-acetyl-D-ribofuranose.

d. Other Compounds of Interest as CDA Inhibitors. In our previous report we indicated the convenience to make structure 12b for the CDA project. The synthesis of the 4-oxoperhydro-1,3-diazepin-2-one aglycone 14 was achieved and the general method of condensation discussed above was used to produce both the N₁-isomer (12a) and the N₃-isomer (structure not shown). This study, in addition, led to the correct assignment of the structure of the natural product squamolone as 1-carbamyl-2-pyrrolidinone (13). Squamolone was originally reported as the diazepinone 14.

1314

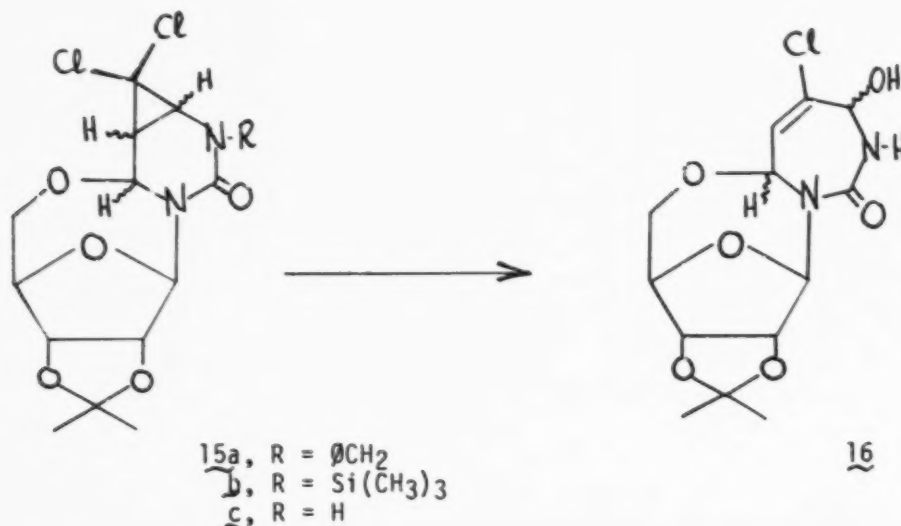
- 12a, R = $\emptyset$ CO, X = O, Y = H
12b, R = H, X = O, Y = H
12c, R = H, X = H,OH, Y = H
12d, R = $\emptyset$ CO, X = H₂, Y = NH₂
12e, R = H, X = H₂, Y = NH₂

Final deblocking of 12a to the final target 12b has met with difficulty. The conditions to deblock the sugar protective groups in the final step also caused cleavage of the 3-4 bond in the aglycon ring. Efforts to circumvent this difficulty are underway together with an alternate synthesis of compound 12c. It should be noted that compound 12c is the ring expanded analog of tetrahydro-uridine (THU).

The amino analog 12e was designed to study the effect of the isosteric replacement of a hydroxyl group in structure 1 by the amino group. This is of particular interest in view of the preferred binding of the enzyme CDA to one of the two diastereoisomers of 1. The target compound 12e was made but it was later found to be contaminated with a ring-contracted product of a still undetermined structure. This work is in progress.

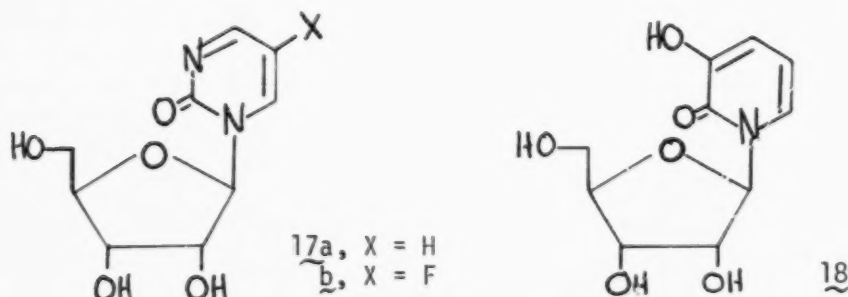
The syntheses of the 2'-deoxy analogues of 1-3 has been temporarily suspended due to difficulties in the separation of isomers.

e. Other Potential CDA Inhibitors Via the Use of Ring Expansion Reactions in Six-membered Nucleosides. The ring expansion approach was reported to be successful in the synthesis of coformycin (M. Ohno *et al.*, *J. Am. Chem. Soc.* 96: 4326, 1974) and was considered as a possible approach to 1 and 3 at the onset of the project. Dr. Beisler of this laboratory also mentioned to us that U.K. Pandit *et al.* (*Heterocycles* 5: 19, 1976) from the University of Amsterdam, had successfully expanded six-membered pyrimidine nucleosides to the corresponding seven-membered ones by a dichlorocarbene insertion reaction followed by thermolysis. A study of this general procedure led us to the design and synthesis of 15a-c,



Compound 15a was found to be very stable and did not ring-expand on heating. Compound 15b hydrolyzed instantly to 15c which spontaneously ring-expanded to 16. Complete characterization of 16 and final conversion to 12c are in progress.

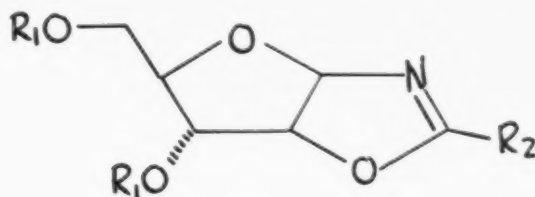
2. In Vivo Active Pyrimidine Nucleosides and Analogues (Dr. Marquez): Compound 17a (NSC 309132), originally studied as a cytidine deaminase inhibitor, was confirmed active in P388. Additional amounts of 17a were prepared in our laboratory and the experimental procedure for 17b was submitted to a Prep Lab for large-scale synthesis.



The fluoro analog (17b, NSC 318509), when tested at a dose regimen similar to that used for 17a, caused excessive weight loss. The compound is currently being tested at lower doses.

The 3-hydroxy-2-pyridone nucleoside 18 (NSC 336233) was prepared and tested. Preliminary *in vitro* testing was not conclusive. Large-scale synthesis of this material is *in progress* for future *in vivo* studies.

3. Alternate Synthesis of Arabinofuranosyl-5-azacytosine (Drs. Numao and Beisler): Oxazoline 19 ($R_1 = t\text{-BuMe}_2\text{Si}$, $R_2 = \text{NH}_2$) and the sulfur analog 20 ($R_1 = \text{H}$, $R_2 = \text{SH}$) were synthesized from arabinose to serve as potential synthetic intermediates for the synthesis of the title compounds. With the correct configuration at C-1 locked in place, it was hoped that the triazine aglycone

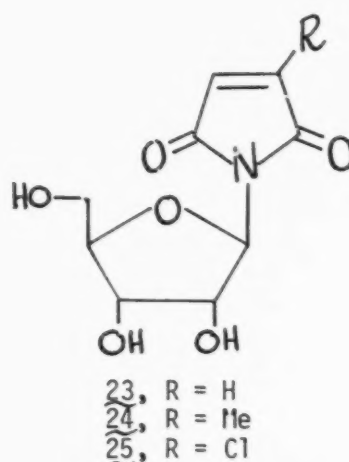
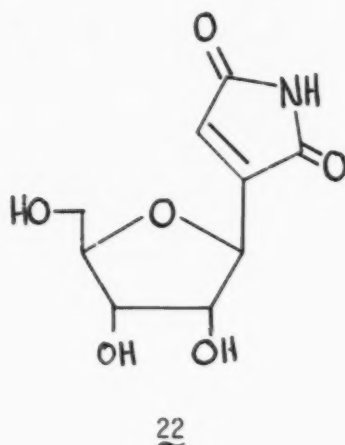
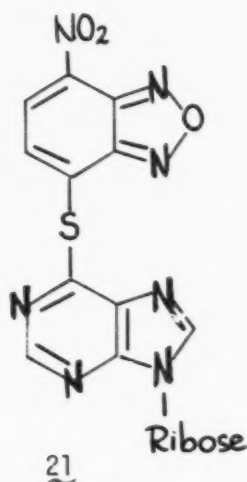


19, $R_1 = t\text{-BuMe}_2\text{Si}$, $R_2 = \text{NH}_2$
20, $R_1 = \text{H}$, $R_2 = \text{SH}$

could be elaborated by a series of condensation reactions. To that end 19 was reacted with R-C(=NH)NH_2 where the leaving group R was MeS, MeO, or imidazo. Starting material was recovered in all cases under a variety of reaction conditions. The exocyclic nitrogen atom has surprisingly poor nucleophilic properties and was found to condense with only very reactive species such as isocyanates. Oxazoline 20 was reacted with guanidine and cyanoguanidine with the intent of nucleophilically displacing the sulfur substituent and giving an intermediate wherein most of the elements of the triazine ring system would be in place. Various reaction solvents, catalysts and reaction conditions failed to provide the desired products.

4. Synthesis of Nucleoside Transport Inhibitors (Drs. Beisler and Rabinovitz): The reagent 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (NBD chloride) readily reacts with thiol and amino groups to give highly fluorescent derivatives which have served in a detection and identification capacity (Birkett et al., *FEBS Letters* 6: 346-348, 1970). Reaction of NBD chloride with nucleosides having exocyclic amino and thiol substituents would provide nucleoside derivatives which bear a structural resemblance to a class of nucleoside transport inhibitors developed by Paterson. Analogs of adenosine, 6-mercaptapurine and 6-thioguanine when N- or S-alkylated with substituted benzyl groups gave a series of potent transport inhibitors (*J. Med. Chem.* 18: 968-973, 1975). It was hoped that condensation of NBD chloride with nucleosides bearing an amino function such as adenosine and cytosine, and thiol nucleosides, such as the riboside and arabinoside of 6-mercaptapurine, as well as 4-thiouridine, 2-thio-6-azauridine and 6-mercaptoguanosine, would yield a novel series of transport inhibitors. Although we have not yet found conditions under which adenosine will

condense with NBD chloride, the chloro substituent of the latter is smoothly displaced by the sulfur atom of 6-mercaptapurine riboside to give compound 21. Evaluation of 21 on the nucleoside transport system has not yet been done, but antitumor activity has been observed for 21 against the P388 leukemia test system.

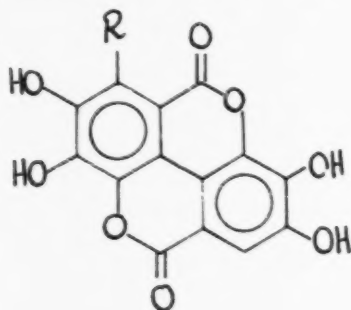


5. Iso-showdomycin Analogs (Drs. Numao, Beisler, Hemmi, Naujokaitis and Rabinovitz): Showdomycin (22) is a C-nucleoside antibiotic which is cytotoxic to cultured HeLa and L1210 cells and shows antitumor activity in the Ehrlich ascites and P388 leukemia test systems. Analogs 23, 24 and 25 of the N-nucleoside types were synthesized and unequivocally given structure assignments using NMR methods, optical rotation observations and chemical conversions. Analogs 23 (and tri-O-acetate), 24 and 25 were tested for antitumor properties using the P388 test system. Although the analogs were quite toxic to mice, no antitumor activity was observed. Tissue culture experiments suggested that the analogs were irreversibly combining with the nucleoside transport carrier protein and consequently were excluding themselves from cellular uptake.

B. Analogs of Antitumor Natural Products

1. Colchicine Derivatives: Synthesis of 4-Substituted Analogs (Dr. Beisler): The 4-formyl, 4-cyano and 4-hydroxymethyl analogs were synthesized and tested for antitumor activity versus P388 leukemia. Although all three compounds showed activity, the 4-formyl analog exhibited greater efficacy than colchicine and was accompanied by indications of reduced toxicity with respect to the parent. The 4-formyl analog was selected for tumor panel testing. Synthetic methods were developed to prepare the 4-chloro, 4-bromo and 4-iodo derivatives. The 4-halo compounds are being synthesized in quantity sufficient for antitumor evaluation. Synthetic methods are under development to provide the 4-nitro, 4-carbomethoxy and 4-acyl analogs.

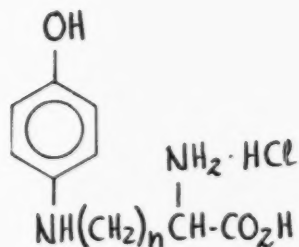
2. Ellagic Acid Derivatives (Dr. Beisler): Ellagic acid, which has a structural similarity to the antitumor antibiotic, chartreusin, was found to have moderate cytotoxicity against cultured L1210 cells and a moderate in vivo activity against P388 leukemia. On the basis of antitumor activity and a uniqueness of structure, ellagic acid (26, R = H) was selected for tumor panel testing. As part of a synthesis program, the tetraacetate and diacetate derivatives of 26 were prepared as synthetic intermediates. One of the objectives in the development of synthetic analogs of 26 was to moderate the very poor solubility of the parent. Oxidative dimerization of gallic acid in concentrated sulfuric acid



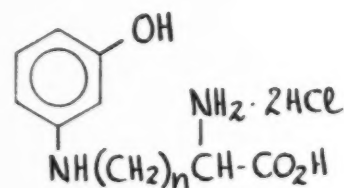
26, R = H
27, R = OH

solution gave hydroxyellagic acid (27, R = OH) in good yield. However, the increase of oxygen substituents in the structure did not lead to a solubility improvement. Hydroxyellagic acid and its pentaacetate derivative were screened for antitumor activity in the P388 test system and were found to be inactive.

C. Design and Synthesis of Agents with Specificity Against Melanotic Melanoma (Dr. Lin): Preferential cell kills against melanotic melanoma was observed among two of the initial target compounds (28 and 29) which we designed to exploit the high tyrosinase activity in melanotic melanoma. The synthesis of three initial target compounds (28-30) were completed with 3 to 4 grams each submitted for in vivo antitumor studies.



28, n = 1
29, n = 3



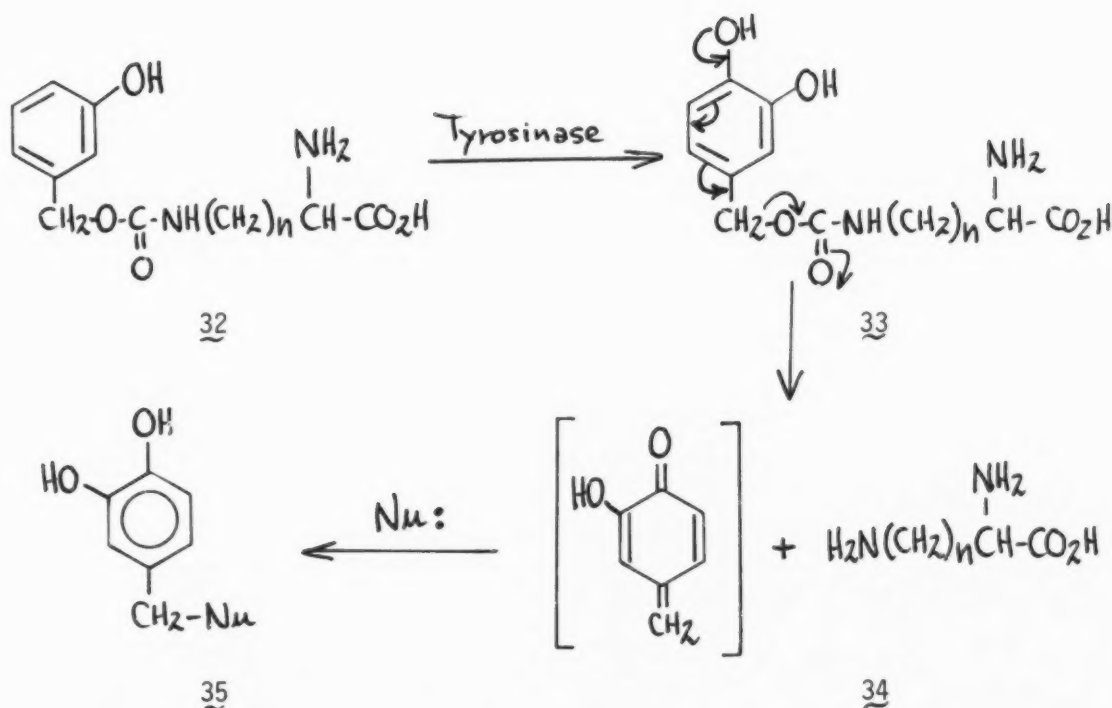
30, n = 3
31, n = 1

Attempts to synthesize compound 31 by acid hydrolysis of its precursor ethyl α -benzamido- β -(m-benzyloxyanilino)propionate, which involved several steps failed, although the same procedure was used successfully to prepare 28.

The synthesis of 29 and 30 was achieved by selective hydrolysis of the protecting functions N-(γ,γ -dicarbethoxy- γ -phthalimido)butyl p- or m-benzyloxyaniline, which in turn was prepared by reductive amination of γ,γ -dicarbethoxy- γ -phthalimidobutyraldehyde with p- or m-benzyloxyaniline and NaBH_3CN . While the free amino acid form is air sensitive, the HCl salts of these new amino acids are relatively stable. However, the high water solubility of HCl-salt required the use of tedious C₁₈ reverse phase column chromatography, a column with limited capacity, to purify these final target compounds.

Preliminary results demonstrated that 28 and 29 possess moderate inhibitory activity against melanotic B16 melanoma in vitro with ID_{50} values of $7.5 \times 10^{-6}\text{M}$ and $4.5 \times 10^{-6}\text{M}$, respectively. A substantially higher concentration is required to inhibit the growth of amelanotic melanoma cells (ID_{50} $7.5 \times 10^{-5}\text{M}$, and $2.5 \times 10^{-5}\text{M}$). No appreciable inhibition was observed for the meta isomer (30) up to 10^{-4}M concentration. These data indicated that compounds 28 and 29 (para-OH isomers) are about 5-10 fold more active against melanotic melanoma, a mutant with high tyrosinase activity, than amelanotic melanoma and that compound 29 is 2-3 times more potent than 28. The difference in activity between 28 and 29 may have been due to the difference in substrate property for tyrosinase or due to difference in transport across cell membrane. Since meta isomer 30 has the same chain length as that of 29 and is a poor inhibitor, the substrate property may be the major factor contributing to the activity of this class of compounds. To attest this possibility, a semiquantitative study on the rate of color development of these new agents upon incubation with tyrosinase was carried out in pH 7.3 tris buffer. It was observed that the order of the rate of color change is as follows: tyrosine > 29 > 28 >> 30. It appears that correlation between in vitro activity against B16 melanoma and substrate property of tyrosinase exists among the series of compounds tested. The inactivity of meta isomer 30 is due to its poor substrate properties for tyrosinase. Development of quantitative methods to measure the rate of hydroxylation and oxidation of these new agents is in progress.

Although the initial targets possess only moderate activity against melanoma in vitro, their preferential inhibition of melanotic melanoma is of particular interest and warrants further synthesis of new analogs to discover more active agents of this class. Compounds with general structure 32 will be the next target compounds. It is envisioned that activation would take place upon hydroxylation of the parent compound 32 by tyrosinase to generate a very reactive intermediate, 2-hydroxy-p-quinomethide (34), which interacts with macromolecules such as protein, DNA and RNA. Two major considerations for the design of compounds with general structure 32 are: (a) substrate property - although compounds with para-OH are generally better substrates than meta-homolog their chemical nature may render the para-OH isomer unstable. Therefore, only the meta-OH derivatives will be designed and synthesized initially; (b) leaving group - since the formation of active and toxic intermediate 34 requires the elimination of the side chain after hydroxylation, the leaving potential of the side chain is of vital importance. To achieve maximal activity, functions with varied leaving potential will be incorporated into the molecule. The synthesis of 32 is currently in progress.



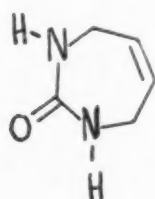
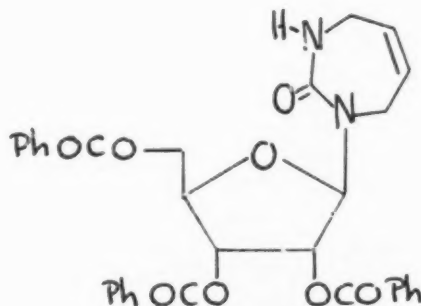
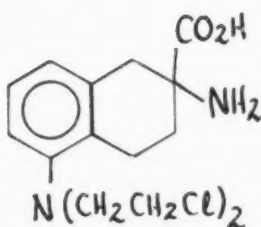
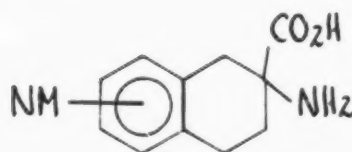
D. Development of New Leads

Catecholamine Analogs as Potential Antitumor Agents (Drs. Lin and Driscoll): Oxidation of a catechol to an o-quinone which then reacted with sulfhydryl containing enzymes was suggested as a possible mechanism of action for this class of compounds in our earlier studies. To further study the structure-activity relationships of catechol analogs and to synthesize compounds with better activity, three new hydroxylated benzylamines, [2,3-dihydroxybenzylamine HI (36), 2,3,4-trihydroxybenzylamine HI (37) and 3,4,5-trihydroxybenzylamine HI (38)], and a series of related compounds were tested against P388 leukemia on the QD 1-9 schedule over the dosage range 600 to 50 mg/kg. New target compounds 36 and 38 are active with T/C (%) equal to 157 and 149, respectively. However, 2,3,4-trihydroxybenzylamine HI (37) is only marginally active with T/C equal to 123 and showed more toxicity than 36 and 38. Again, the generalized structure-activity relationships described previously for the catechol amines continue to apply in the benzylamine series. Positioning of vicinal hydroxyl groups in the benzylamine series such that oxidation to ortho quinoid derivatives might occur, provided active materials such as 3,4-dihydroxybenzylamine, 3,4,5-trihydroxybenzylamine, 2,3,4-trihydroxyphenyl methyl ketone, 2,3-dihydroxybenzylamine and 3,4-dihydroxybenzaldehyde. Dihydroxy compounds which cannot be oxidized to a quinoid form such as 2,4-dihydroxybenzaldehyde or can be oxidized only to a para-quinone, such as 2,5-dihydroxybenzaldehyde are inactive. Further testing for activity against B16 melanoma is currently in progress.

E. Prep Lab (Mr. Fuller, Drs. Marquez and Driscoll)

The Drug Design and Chemistry Section devotes a one-man per year effort in synthetic organic and HPLC analytical support devoted to requests for chemical assistance from various parts of DCT. Much of the work is in response to biochemical investigator's requests for specific non-commercially available molecules. Providing this service is in keeping with the Section's role in the interdisciplinary aspects of the LMCB. Since most of the requests are for compounds which are either unknown or inadequately described in the literature, considerable research usually is required before a target compound can be prepared and completely characterized.

Very pure tetrahydrouridine was prepared by preparative HPLC methods for cytidine deaminase inhibition studies. Compounds 39-42 are typical of the molecules prepared. Ten grams of 39 were prepared as a precursor for 40. The synthesis of 39 is significant because gram amounts of deblocked 40 are required for in vivo combination experiments with ara-C and 5-azacytidine. Deblocked 40 is the best inhibitor of human liver cytidine deaminase known. Compounds 41 and 42 were the products of a multi-step synthesis. They were prepared to test an amino acid antitumor agent transport hypothesis for Dr. Vistica, a LMCB biochemist. The position of nitrogen mustard substitution in 41 was determined by X-ray analysis.

394041

NM = N(CH₂CH₂Cl)₂ in
6- or 7-position

42

Publications:

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Lin, A.J. and Driscoll, J.S.: Catecholamine analogs as potential antitumor agents II. J. Pharm. Sci., in press.

Numao, N., Hemmi, H., Naujokaitis, S.A., Rabinovitz, M., and Beisler, J.A.: Showdomycin analogs: Synthesis and antitumor evaluation. J. Med. Chem., in press.

Marquez, V.E., Liu, P.S., Driscoll, J.S. and McCormack, J.J.: Cyclic urea nucleosides. Cytidine deaminase activity as a function of aglycon ring size. J. Med. Chem., in press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03581-12 LMCB								
PERIOD COVERED October 1, 1980 to September 30, 1981										
TITLE OF PROJECT (80 characters or less) The Analytical Chemistry of New Anticancer Drugs										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: J. A. Kelley</td> <td style="width: 33%;">Research Chemist</td> <td style="width: 15%;">LMCB</td> <td style="width: 19%;">NCI</td> </tr> <tr> <td>Other: J. S. Driscoll</td> <td>Head, Drug Design and Chemistry Section</td> <td>LMCB</td> <td>NCI</td> </tr> </table>			PI: J. A. Kelley	Research Chemist	LMCB	NCI	Other: J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI
PI: J. A. Kelley	Research Chemist	LMCB	NCI							
Other: J. S. Driscoll	Head, Drug Design and Chemistry Section	LMCB	NCI							
COOPERATING UNITS (if any) Medicine Branch, COP, DCT, NCI; Laboratory of Clinical Biochemistry, BCRP, DCT, NCI										
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology										
SECTION Drug Design and Chemistry Section										
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The objective of this project is the research and development of analytical methods which are used to: (1) establish the <u>structure and purity</u> of new <u>antitumor agents</u> and their <u>metabolites</u> , (2) determine <u>physical and chemical</u> properties of new anticancer drugs, (3) quantitate drugs and their metabolites in physiological samples to elucidate <u>pharmacology</u> and to determine <u>pharmacokinetics</u> and, (4) study <u>reaction mechanisms</u> of potentially useful <u>synthetic transformations</u> . <u>Mass spectrometry</u> , <u>gas chromatography</u> and <u>high-performance liquid chromatography</u> are emphasized techniques. Compounds of current interest are <u>aziridinybenzoquinones</u> , <u>reduced pyrimidine nucleosides</u> , <u>seven-member ring nitrogen heterocycles</u> , <u>nitrogen mustards</u> and <u>cytidine deaminase inhibitors</u> . The acid and base-catalyzed <u>rearrangements</u> of <u>pyrimidine nucleosides</u> are under investigation.										

Project Description:General Objectives:

The objective of this project is the research and development of analytical techniques which are used to:

1. establish the structure and purity of new anticancer drug candidates;
2. establish structures of metabolites of new antitumor agents;
3. determine important physical and chemical properties of new anticancer drugs;
4. study reaction mechanism;
5. quantitate drugs and their metabolites in physiological samples.

Methods Employed:

Mass spectrometry (MS), gas-liquid chromatography (GC) and the combination of these techniques (GC/MS) are emphasized. Other analytical methods such as high pressure liquid chromatography (HPLC), NMR, UV and IR spectroscopy also are employed.

Major Findings:I. Research ProblemsA. Clinical Support and Pharmacology Studies

1. HPLC Analysis of AZQ in Biological Media (Drs. Kelley and Chong): The high-performance liquid chromatography (HPLC) assay for AZQ was further refined and applied to the determination of the parent drug in rat, dog and human samples. A single chloroform extraction of the biological matrix gave AZQ recoveries of greater than 88% from plasma, urine and CSF in the range of expected physiological concentrations (20-800 ng/ml). Isocratic reverse phase HPLC with UV detection at 340 nm resulted in a limit of detection of approximately 3 ng/ml and an average relative standard deviation of 5.5% in replicate analyses. The assay was then applied to relate earlier data based on radioactivity to directly measured AZQ levels. A correlation coefficient of 0.996 was calculated when chloroform-extractable plasma radioactivity and HPLC-determined AZQ plasma concentrations from rats administered 1 mg/kg ^{14}C -AZQ were directly compared. The pharmacokinetic parameters were also the same for rats given a bolus iv dose of either unlabeled or ^{14}C -AZQ with a two-compartment open model explaining the plasma drug disappearance curve. A very rapid initial redistribution phase with $t_{1/2} = 3.2$ min was followed by a plasma elimination phase with a half-life of 26.5 min.

The situation for renal excretion of the drug was quite different, however. In dogs very little of the chloroform-extractable radioactivity was unchanged AZQ and this was only a small fraction of the total urinary radioactivity. Low levels of 24-99 ng/ml of parent drug could be detected in the first three hourly individual urine collections from several dogs, but the HPLC profile of

radioactivity indicated five and perhaps as many as eight as yet unidentified metabolites or degradation products. AZQ was undetectable in later urine samples although radioactivity persisted. HPLC analysis also indicated that the efficiency of ultrafiltration for AZQ recovery was 91% in the range 0.1-1.0 $\mu\text{g/ml}$. Thus about 8-10% of the drug is bound to rat plasma protein at this concentration level.

2. AZQ Phase I Clinical Trial (Drs. Kelley and Schilsky, MB-COP): Forty previously treated patients with advanced cancer were given 1-25 mg/m^2 of AZQ as a 20-minute iv infusion on days 1 and 8 of a 28-day cycle. The HPLC assay described above was used to measure AZQ in the plasma, urine or CSF of 20 of these patients. AZQ plasma pharmacokinetics could be determined in 11 different patients receiving drug doses ranging from 1 to 20 mg/m^2 . All subjects showed a very rapid redistribution phase ($t_{1/2}(\alpha) = 2.8 \pm 1.3$ min) followed by a slower yet still fast plasma elimination phase. The mean elimination half-life for the terminal phase of the two-compartment open model to which the data was fitted was 33.3 ± 4.5 min and was dose independent. The pharmacokinetics in man closely paralleled those in the rat, dog and rhesus monkey. A mean total body clearance of 517 ± 155 ml/min implied the involvement of hepatic as well as renal clearance. The apparent volume of distribution at steady state averaged 15.8 ± 4.0 liters, suggesting a distribution only slightly less than the total extracellular water compartment.

The urinary excretion of unchanged AZQ was examined in 5 patients receiving 10 mg/m^2 or more of the drug. Measurable amounts of intact drug could be found in only two of these patients and represented less than 0.2% of the total administered dose. Cerebrospinal fluid, obtained via lumbar puncture was available from 3 different patients, including one from two different dosage cycles. The table below shows that AZQ enters the CSF and achieves concentrations that are substantial compared to plasma levels:

Patient	Dose (mg/m^2)	Sample Time (min)	[AZQ] _{plasma} (ng/ml)	[AZQ] _{CSF} (ng/ml)
F.S.	15	90	51	72
P.C.	17.5	65	162	88
T.S.	20 ^a	60	292	61
T.S.	20 ^b	95	110	61

a. Day 1, Cycle 1

b. Day 8, Cycle 3

This confirms that AZQ functions as originally postulated in its synthetic design and enters the central nervous system. Although no measurable antitumor responses were noted during this study, 3 patients with malignant gliomas had objective neurologic improvement. A more detailed study of AZQ metabolism and pharmacokinetics is planned for the ensuing Phase II clinical trial in the Medicine Branch.

3. AZQ Phase I/II Clinical Trial (Drs. Kelley, Van Echo and Bachur, BCRP): Patients with leukemias were treated with AZQ given by a 30 min iv infusion on a daily X 7 schedule at a starting dose of 16 mg/m². Since these patients had Omayra reservoirs implanted before the start of the study, serial plasma and CSF samples were available for pharmacokinetic analysis. The following table summarizes the preliminary kinetic data obtained from the first 3 patients in this study by using the HPLC assay developed in this laboratory:

Patient	Dose (mg/m ²)	Plasma		CSF	
		t _{1/2} (α) (min)	t _{1/2} (β) (min)	t _{1/2} (min)	[AZQ] _{max} (ng/ml)
S.S.	16	4.0	24.9	89.1	89
M.W.	16	4.5	32.0	80.0	170
L.Q.	17.5	4.0	30.8	52.8	253

The AZQ plasma disappearance curve was fitted to a two-compartment open model and the calculated pharmacokinetic parameters were within the range of previous studies. Maximum CSF levels were attained 45-60 min after the end of infusion and they equaled or exceeded the corresponding plasma concentrations at this time. Clearance of the drug from CSF was much slower than from plasma; AZQ could still be detected in the CSF after 8 hr. A preliminary calculation of a partition coefficient (P) from CSF and plasma data gave a log P = -0.24; this is within the range of the experimentally measured log P = 0.05 in the octanol/water system.

B. Furanose-Pyranose Interconversion (Drs. Kelley and Marquez): The pentose sugars of certain perhydropyrimidine and 1,3-diazepin-2-one nucleosides which did not possess extended conjugation in the base were easily isomerized from a furanose to pyranose configuration (e.g. 1 to 2). Since the pyranose analogs were much less potent cytidine deaminase inhibitors than the furanose compounds, control of this isomerization was essential for synthesis of the most active inhibitors. This unusual acid-catalyzed furanose to pyranose ring expansion of reduced nucleosides was further studied and characterized using GC/MS and NMR techniques. Deuterium labeling was employed to elucidate the mass spectral fragmentation patterns of the trimethylsilyl derivatives of these isomers and to confirm that the proposed isomerization was occurring. Proton NMR was used to show that the preferred pyranose configuration was the one in which 3 of the 4 bulky substituents (i.e. hydroxyls and aglycon) were equatorial with H¹ and H² trans-diaxial. GC and HPLC methods were developed to assess the degree of isomerization and to isolate the pure material of each analog. The isomerization was found to be a rapidly occurring equilibrium where 80-85% of the β -furanose nucleoside was converted to the β -pyranose isomer at room temperature.

II. Synthetic and Collaborative Project Support (Dr. Kelley)

1. Structure Determination of Squamolone: The natural product squamolone, 1-carbamoyl-2-pyrrolidinone (3), was originally reported to be 4-oxoperhydro-1,3-diazepin-2-one (4). Direct probe mass spectral analysis, deuterium labeling and metastable ion analysis of these two isomers did not allow structural differentiation. However, silylation of squamolone formed only a mono-trimethylsilyl derivative because of strong intermolecular hydrogen bonding. Heating to force complete silylation resulted in decomposition to 2-pyrrolidinone. Compound 4 formed the expected bis-trimethylsilyl derivative.

2. Perhydro-1,3-diazepin-2-ones: The nucleosides of this class of compounds, which can be thought of as reduced and ring expanded pyrimidines, are of much interest as potent cytidine deaminase inhibitors. Synthetic intermediates and target nucleosides were characterized by gas chromatography, mass spectrometry and combined GC/MS after suitable derivatization in a continuation of previous work.

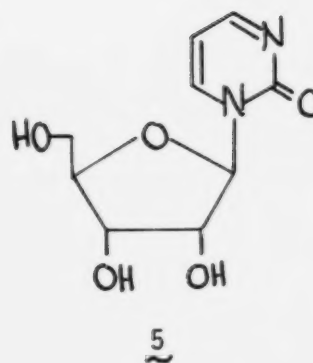
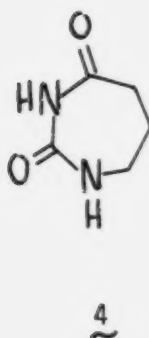
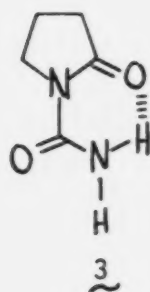
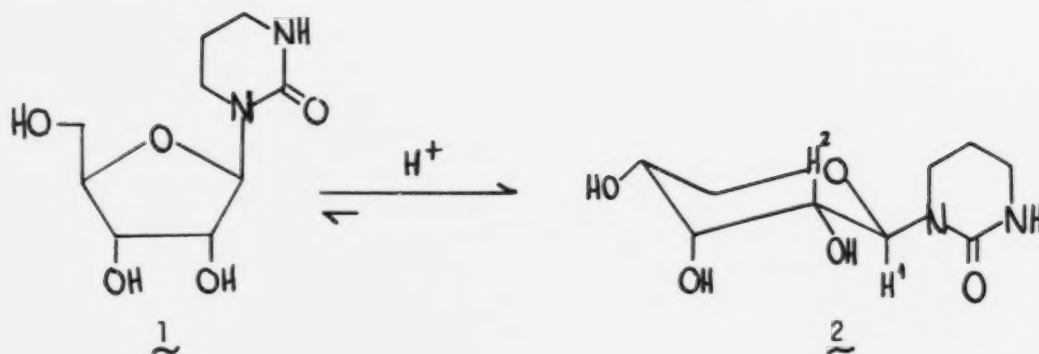
3. 2-Pyrimidinone Rearrangement: Treatment of riboside 5, an inhibitor of DNA synthesis, with strong base followed by strong acid gave a high yield of a rearranged product. Mass spectral analysis of both underivatized and silylated sample indicated a probable molecular weight of 175 with three sites of silylation. Although the structure of this intriguing rearrangement product could not be unequivocally determined, the ribose sugar was no longer present and a portion of the base was lost while the UV adsorption both increased and shifted to lower wavelength.

4. Miscellaneous: Numerous samples which cannot be categorized as coming from one of the above project areas were analyzed by the appropriate mass spectral and chromatographic techniques on an individual basis. Included in this group were anhydronucleosides, isopropylidene nucleosides, nucleosides of cyclic ureas, underivatized pyrimidine nucleosides, tetrahydrouridine and its thermal decomposition products, 2-aminotetrahydronaphthoic acid mustards, colchicine derivatives, showdomycin analogs, phthalates, phthalimide-protected amino acid derivatives, hydroxyanilino amino acid derivatives, and a polycyclic phenolic lactone.

Publications:

Kelley, J.A., Abbasi, M.M. and Beisler, J.A.: Silylation-mediated oxidation of dihydropyrimidine bases and nucleosides. Anal. Biochem. 103: 203-213, 1980.

Poochikian, G.K. and Kelley, J.A.: 2,5-Diaziridinyl-3,6-bis(carboethoxyamino)-1,4-benzoquinone: II. Isolation and characterization of degradation products. J. Pharm. Sci. 70: 162-167, 1981.



SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03582-07 LMCB																
PERIOD COVERED October 1, 1980 to September 30, 1981																		
TITLE OF PROJECT (80 characters or less) Structure-Activity Studies of Anticancer Drugs																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: F. R. Quinn</td> <td style="width: 33%;">Chemist</td> <td style="width: 15%;">LMCB</td> <td style="width: 19%;">NCI</td> </tr> <tr> <td>Other: J. S. Driscoll</td> <td>Head, Drug Design and</td> <td></td> <td></td> </tr> <tr> <td></td> <td>Chemistry Section</td> <td>LMCB</td> <td>NCI</td> </tr> <tr> <td>J. A. Beisler</td> <td>Research Chemist</td> <td>LMCB</td> <td>NCI</td> </tr> </table>			PI: F. R. Quinn	Chemist	LMCB	NCI	Other: J. S. Driscoll	Head, Drug Design and				Chemistry Section	LMCB	NCI	J. A. Beisler	Research Chemist	LMCB	NCI
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COOPERATING UNITS (if any) Drug Evaluation Branch, DTP, DCT, NCI; Pomona College; University of Vermont																		
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology																		
SECTION Drug Design and Chemistry																		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205																		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER:																
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																		
SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are: (1) the development of quantitative <u>structure-activity</u> correlations among <u>antitumor agent</u> congeners, (2) the development of general <u>structure-activity</u> relationships among large numbers of unrelated antitumor agents and (3) the use of the correlation data obtained to generate leads for new synthesis and increase the rationale for the testing of new agents. Compound families of current interest are <u>colchicines</u> , <u>purines</u> , <u>2,4-diaminopyrroloquinazolines</u> , <u>quinoliziniums</u> , <u>benzimidazole mustards</u> , <u>phenanthridiniums</u> , <u>Schiff-base alkylating agents</u> and <u>thiadiazoles</u> .																		

Project Description:General Objectives:

The objectives of this project are:

1. the development of quantitative correlations of antitumor and toxicity data with structural features among antitumor agent congeners;
2. the investigation of structure-activity techniques as applied to antitumor data;
3. the development of correlations between structure and antitumor activity among large numbers of structurally unrelated compounds;
4. the use of the correlations obtained to: (a) generate leads for new synthesis, (b) increase the rationale for selection of new compounds to be tested and (c) reduce the number of synthetic analogs necessary to obtain the optimally active member of a new series.

Methods Employed:

Computer techniques are used to analyze and organize biological screening data. These data are then correlated with molecular structural parameters using computerized regression analysis techniques.

Major Findings:I. Quantitative Structure-Activity Relationships by the Hansch TechniqueA. Intramural Studies (Dr. Quinn)

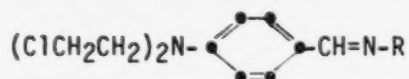
1. Colchicine Analogs: Toxicity and Activity Studies (Drs. Beisler, Neiman, Quinn): Virtually all structural modifications of this series have been made at the 7- and/or 10-positions of the molecule. A study of the effect of structural variations on the *in vivo* P388 activity of these compounds has been completed and published. A study of the acute toxicity of this series has been completed and submitted for publication. The toxicity and activity correlations are parallel and show the same dependence on $\log P$, $(\log P)^2$ and the positive effect of electron-withdrawing groups on the amide nitrogen. The optimal $\log P$ values for both activity and toxicity are quite close (1.17 vs. 1.19). Thus, modification at those positions to decrease toxicity is likely to result in a decrease in potency and vice versa.

In the course of this investigation, it was found that A-ring-modified colchicines do not obey the QSAR relationships which were established for 7- and 10-modified analogs. The 4-formyl, 4-cyano and 4-(hydroxymethyl) compounds were synthesized to test this hypothesis. *In vivo* P388 data show that the formyl derivative is both less toxic and more potent than the parent. This compound has been placed in the tumor panel.

2. Purines: A correlation between pK_a and standard aromatic electronic parameters (σ_m , σ_p) was achieved for 2-, 6- and 8-substituted purine bases and the results have been submitted for publication. These findings indicate that purines are amenable to QSAR treatment without the determination of special parameters. Work is proceeding on the correlation of toxicity (LD_{50}) and P388, L1210 and CA755 activity of 2-, 6- and 8-substituted purines.

3. 2,4-Diaminopyrroloquinazolines: A cooperative project with Dr. John McCormack (University of Vermont) is continuing. Additional compound data have been added to the correlation of the enzyme inhibition of *C. oncopelti* dihydrofolate reductase. The enzyme inhibition shows a parabolic dependence on $\log P_1(\log P_0) = 2.5$.

4. Schiff Base Alkylating Agents: Work is proceeding on a correlation of activity and toxicity of alkylating agents having the general structure:



5. 5-Aminothiadiazoles. This series of congeners is being examined with a view to establishing a relationship between antitumor activity and toxicity and structural parameters.

6. Anthracenediones: This series of compounds was examined in cooperation with Prof. Hansch. It failed to yield a satisfactory QSAR correlation, mainly due to the presence of limited structural variation.

7. Anderson Pyrrolizines: These congeners were examined and failed to yield a correlation. The intractability of this series is probably also due to limited structural variations.

8. Data Analysis Techniques: A method for deriving a tumored LD_{50} from toxicity day survivors has been devised and tested for colchicine analogs against LD_{50} 's derived from AA testing. The following relationship was derived

$$\log(1/LD_{50})_{\text{tumored}} = 0.63(\pm 0.58) + 0.92(\pm 0.12) \log(1/LD_{50})_{\text{untumored}}$$

$$n = 10; \quad r = 0.974; \quad s = 0.140$$

where the LD_{50} is in units of moles/kg. This technique is being tested in other series and, if the relationship holds, should provide an inexpensive method of deriving toxicity for use in the early stages of drug design and testing.

B. Contract Studies (Drs. Hansch, Quinn)

1. Anthracyclines: A study of the relationship of activity and cardiotoxicity to structural parameters has been completed and published. The activity of anthracyclines against ip B16 melanoma yielded the following correlation:

$$\log(1/C) = -0.41(\pm 0.13)\log P + 0.48(\pm 0.35)I_0 + 0.81(\pm 0.38)I_1 + 6.57(\pm 0.32)$$

$$n = 23; \quad r = 0.974; \quad s = 0.288$$

In this equation I_0 is given the value of 1 for congeners in which the 4-OCH₃ has been converted to OH or H. I_1 is assigned a value of 1 for a set of very lipophilic hydrazones. These hydrazones greatly increase activity, other factors being constant.

The Zbinden data for cardiotoxicity yielded the following correlation:

$$\log(1/C) = -0.30(\pm 0.11)\log P + 1.01(\pm 0.25)I_0 + 0.69(\pm 0.33)I_1 + 0.74(\pm 0.34)I_2 + 4.82 (\pm 0.22)$$

$$n = 21; \quad r = 0.934; \quad s = 0.181$$

In this correlation, I_0 and I_1 have the same meaning as in the activity correlation. I_2 is assigned the value of 1 for N-alkylation of the amino sugar moiety. This N-alkylation does not apparently alleviate cardiotoxicity.

In general, cardiotoxicity and activity appear to be highly correlated. The introduction of small hydrophilic electron-releasing groups in the A-ring which are conjugated with the carbonyl function promise a greater reduction in cardiotoxicity than antitumor activity.

Publications:

Fink, S.I., Leo, A., Yamakawa, M., Hansch, C., and Quinn, F.R.: The quantitative structure selectivity relationship of anthracycline antitumor activity and cardiac toxicity. Il Farmaco, Sci. Ed. 35: 965-973, 1980.

Quinn, F.R. and Beisler, J.A.: Quantitative structure-activity relationships of colchicines against P388 leukemia in mice. J. Med. Chem. 24: 251-256, 1981.

Quinn, F.R., Neiman, Z. and Beisler, J.A.: Toxicity quantitative structure activity relationships of colchicines. J. Med. Chem., in press.

Neiman, Z. and Quinn, F.R.: Quantitative structure-activity relationships of purines I: Choice of parameters and prediction of pK_a 's. J. Pharm. Sci., in press.

Applied Pharmacology Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1980 - September 30, 1981

The objectives of the Applied Pharmacology Section are to conduct biochemical and molecular pharmacological studies of anticancer drugs under development by the Division of Cancer Treatment, with emphasis on agents arising from within LMCB.

At present, the Section is primarily engaged in biochemical studies of nucleoside anticancer agents. The first project deals with the adenosine deaminase inhibitor, 2'-deoxycoformycin (dCF). This agent has proved useful for investigating the mechanism of cytotoxicity resulting from blockade of adenosine deaminase, an experimental condition mimicking the severe combined immunodeficiency syndrome in patients. dCF also possesses immunosuppressive properties and is currently in Phase II clinical trials. This Section has assessed the pharmacokinetics of dCF in the plasma, and the levels of adenosine metabolites in the plasma and urine of acute leukemic patients. Experimental studies are also underway investigating the action of dCF in vitro on T and B lymphocyte cultures from mouse spleen utilizing specific mitogens.

The second project entails detailed investigations of the synthesis, methylation and function of RNA in response to nucleoside anticancer drugs. These studies have included both a cell kinetic and biochemical analysis of the action of the adenosine analog antibiotic, sangivamycin. This drug has proved to be one of the most potent adenosine analogs yet tested, and is believed to exert its highly time-dependent toxicity via effects on RNA synthesis per se. Initial studies with mouse sarcoma 180 and Ehrlich ascites cells have now been extended to human colon carcinoma cells in culture.

In conjunction with the latter studies, the ability of the antimetabolites, 5-fluorouracil, 5-azacytidine and dihydro-5-azacytidine to modify the translational activity of messenger RNA has been assessed in vitro using cell-free translation systems. The capacity of these drugs to induce miscoding has been postulated for some time, but we have discovered that this effect is not one of their primary modes of action.

The last project is concerned with the relationship between the phosphorylation of specific classes of nonhistone chromosomal proteins and the inhibitory activity of adenosine analogs on transcription. We are currently examining the high mobility group (HMG) class of nuclear proteins which are intimately associated with gene activity in the transcriptional structural unit, the nucleosome. We have discovered that two of the four HMG proteins (HMG 14 and 17) are highly phosphorylated, and that these phosphorylated species are associated with transcriptionally active regions of chromatin. The phosphorylation of HMG 14 and 17, however, is not affected by several pyrrolopyrimidine analogs including sangivamycin despite their pronounced inhibitory effects on nuclear RNA synthesis.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 06145-04 LMCB																									
PERIOD COVERED October 1, 1980 to September 30, 1981																											
TITLE OF PROJECT (80 characters or less) Characterization of Adenosine Deaminase Inhibitors in Normal and Neoplastic Tissues																											
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COOPERATING UNITS (if any) Pediatric Oncology Branch, DCT, NCI																											
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology																											
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TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.6	OTHER:																									
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS																											
SUMMARY OF WORK (200 words or less - underline keywords) The mechanism of action of the immunosuppressive effect of dCF is being determined <u>in vitro</u> with <u>T and B lymphocyte</u> cultures from mouse spleen and thymus. Measurements of <u>adenosine metabolites</u> , and DNA and RNA synthesis will be correlated with the suppression of blastogenesis by dCF resulting from the mitogenic response of the cells to <u>concanavalin A</u> and <u>lipopoly-saccharide</u> . The metabolism of dCF and its incorporation into nucleic acids will also be assessed in ConA-stimulated spleen cells.																											

Objectives:

Adenosine deaminase plays a central, but passive role in maintaining the viability of cells. Suppression of its activity by inhibitors such as 2'-deoxycoformycin (dCF) or by its genetic deletion in the severe combined immunodeficiency syndrome results in the inability of lymphocytes to function normally in their immune response. This is presumably due to the build up of adenosine metabolites such as ATP, dATP, cyclic AMP or S-adenosylhomocysteine, which are potent inhibitors of various metabolic processes if allowed to accumulate to inhibitory concentrations within the cell.

The ability of dCF to potentiate the inhibitory effects of adenosine analogs on RNA synthesis, cytotoxicity and the phosphorylation of nuclear proteins has previously been studied within this Section. We are currently investigating the direct effect of dCF in vitro on T and B lymphocytes on the suppression of blastogenesis, nucleic acid synthesis, and formation of adenosine metabolites such as dATP. In addition, we are examining the relevance of the incorporation of dCF into DNA with respect to its inhibitory effect on DNA synthesis.

Methods Employed:

Cultures of mouse spleen cells in RPMI 1640 and measurement of blastogenesis after addition of concanavalin A and lipopolysaccharide by incorporation of [³H]thymidine and [³H]uridine. Separation of nucleotide metabolites of dCF by DEAE Sephadex-urea chromatography with a linear gradient of 0.1 - 0.7 M NaCl in 20 mM TRIS (pH 7.9). Isolation of DNA by detergent extraction and CsSO₄ gradient centrifugation. Base analysis of DNA by DNase, phosphodiesterase and alkaline phosphatase digestion followed by reversed phase HPLC using 10 mM KH₂PO₄ (pH 3.65):5% methanol.

Major Findings:

1. dCF, a potent inhibitor of adenosine deaminase was tested in a Phase I clinical trial and was found to be therapeutically active in acute lymphoblastic leukemia. Levels of dCF in plasma, plasma concentrations of adenosine and deoxyadenosine, and urine levels of deoxyadenosine were measured in 19 of 26 leukemic patients undergoing treatment with dCF. dCF was administered intravenously to 26 patients at a dose of 0.25 to 1.0 mg/kg (7.5 to 30 mg/m²) for three consecutive days. Dose-limiting toxicity (renal and central nervous system) was observed at 0.75 to 1.0 mg/kg of dCF, and was associated with peak plasma drug levels of 4 to 6 μ M. The plasma concentrations of adenosine and deoxyadenosine and the urine concentration of deoxyadenosine did not correlate with dCF dose, therapeutic response or toxicity.

Proposed Course:

In vitro studies of dCF on mouse spleen cells will be completed. We presently have evidence that dCF is incorporated into DNA of ConA-stimulated spleen lymphocytes, and we are determining whether this effect is related to inhibition of DNA synthesis during drug-sensitive and drug-resistant periods of mitogen stimulation. Application of these findings to lymphoblasts of acute leukemic patients and a human T cell lymphoma cell line in culture are planned.

Publications:

Glazer, R.I.: 2'-Deoxycoformycin and other adenosine deaminase inhibitors. Rev. Drug Metab. III: 105-128, 1980.

Glazer, R.I.: Adenosine deaminase inhibitors: Their role in chemotherapy and immunosuppression. Cancer Chemother. Pharmacol. 4: 227-235, 1980.

Venner, P.M., Glazer, R.I., Blatt, J., Sallan, S., Rivera, G., Holcenberg, J.S., Lipton, J., Murphy, S.B. and Poplack, D.G.: Clinical Pharmacology of 2'-deoxycoformycin: Levels of 2'-deoxycoformycin, adenosine and deoxyadenosine in patients with acute lymphoblastic leukemia. Cancer Res. (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07109-05 LMCB																									
PERIOD COVERED October 1, 1980 to September 30, 1981																											
TITLE OF PROJECT (80 characters or less) Effect of Anticancer Drugs on the Synthesis and Function of Nuclear RNA																											
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SUMMARY OF WORK (200 words or less - underline keywords) The synthesis and processing of nuclear and cytoplasmic <u>transfer RNA (tRNA)</u> , <u>ribosomal RNA (rRNA)</u> and <u>messenger RNA (poly(A)RNA)</u> will be studied in <u>LT2T0</u> and <u>Ehrlich ascites cells</u> . The effects of the antimetabolites: <u>5-fluorouracil</u> , <u>5-azacytidine</u> , <u>dihydro-5-azacytidine</u> , <u>sangivamycin</u> , and <u>xylosyladenine</u> will be examined on the methylation and synthesis of <u>rRNA</u> , <u>tRNA</u> and <u>poly(A)RNA</u> . 5-Azacytidine and 5-fluorouracil are believed to also exert their antitumor effects through their incorporation into mRNA. This hypothesis will be tested by quantitative and qualitative assessment of the capacity of polysomal poly-(A)RNA from Ehrlich ascites cells to code for proteins in heterologous cell-free <u>translation systems</u> derived from <u>wheat germ extracts</u> and <u>reticulocyte lysates</u> . These studies will include analyses of the drug-modified poly(A)RNA by agarose gel electrophoresis, and analysis of translation products by polyacrylamide slab gel electrophoresis and autoradiography.																											

Objectives:

Many nucleoside anticancer drugs act by inhibiting the transcription and/or processing (via methylation) of ribosomal RNA (rRNA), transfer RNA (tRNA) and messenger RNA (poly(A)RNA). The specific modifications of these RNA's involves both base and 2'-O-methylation, and in some instances are more sensitive to inhibition than the transcription of the RNA itself. The main objective of this area of research is to assess the specificity of nucleoside anticancer drugs such as xylosyladenine on both transcription and the aforementioned methylation reactions for several classes of nuclear and polysomal RNA.

Antimetabolites such as 5-fluorouracil (5-FU) and 5-azacytidine (AZC) are also believed to exert their antitumor effects by being incorporated into mRNA and initiating miscoding during translation. To test this hypothesis, poly(A)RNA will be isolated from polyribosomes of Ehrlich ascites cells incubated in vitro and analyzed in an in vitro translation system prepared from wheat germ extract or reticulocyte lysate. The labeled translation products will be analyzed by polyacrylamide gel electrophoresis. In addition, poly(A)RNA will be analyzed for modification of its size, and rate of synthesis resulting from treatment with 5-FU and other anticancer drugs. Hence the objectives of this project are to study the action of antimetabolite anticancer drugs such as 5-FU upon both the transcriptional and post-transcriptional processes associated with RNA function in tumor cells, and to relate these changes to their antitumor activities.

Methods Employed:

Isolation of nuclei from L1210 and Ehrlich ascites cells by Triton X-100 lysis and sucrose gradients; labeling of nRNA with radioactive precursors such as [³H]adenosine, [¹⁴C]uridine and [³H-methyl]L-methionine; extraction of nRNA by the use of SDS-buffer-phenol extraction; characterization of nRNA via poly(U)-Sephacrose affinity chromatography, 2% agarose-urea-SDS-iodoacetate gel and 8% polyacrylamide gel electrophoresis; alkaline hydrolysis and DEAE Sephadex-urea chromatography; separation of methylated bases via two-dimensional TLC; measurement of UTP and S-adenosyl-L-methionine (SAM) specific radioactivities and concentrations by HPLC on anion exchange and C¹⁸ columns, respectively. Isolation of polyribosomes by Mg⁺⁺ precipitation of 10,000 x g extracts of rat liver or Ehrlich cells; extraction of poly(A)RNA by SDS-phenol-HCCl₃ and poly-(U)-Sephacrose chromatography; incubation of poly(A)RNA with [³H]leucine or [³⁵S]methionine, amino acids, KCl, GTP, ATP, PEP, Mg⁺⁺, spermidine and wheat germ extract or reticulocyte lysate; extraction of labeled proteins, and product analysis on polyacrylamide slab gels using fluorography; measurement of the size distribution of poly(A)RNA by agarose-urea-SDS-iodoacetate gel electrophoresis.

Major Findings:

1. The biological effects of N-(phosphonacetyl)-L-aspartate (PALA) and 5-FU were examined singly, and in combination on the growth of a human mammary carcinoma (MDA) cell line in culture. All combinations of 5-FU (2.5×10^{-7} to 1.5×10^{-5} M) and PALA (6.0×10^{-5} to 3.6×10^{-3} M) resulted in synergistic inhibition of cell growth as revealed by a 50% response isobologram. To examine

the biochemical basis for the synergism, measurements of the incorporation of [^3H]5-FU into total non-poly(A)- and poly(A)- RNA, and simultaneous incorporation of [^{14}C]deoxyguanosine and [^3H]deoxyuridine into DNA were determined. The combination of 3.7×10^{-5} M PALA and 1×10^{-6} M 5-FU produced 65-85% inhibition of cell growth after continuous treatment for one to three days. Treatment of the cells for 3 or 24 hr with the same drug regimen produced approximately a 170% increase in the incorporation of 1×10^{-6} M [^3H]5-FU into poly(A)RNA in comparison to [^3H]5-FU treatment alone; exposure for 24 hr with 3.7×10^{-5} M PALA and 1×10^{-6} M [^3H]5-FU resulted in a 285% increase in the incorporation of [^3H]5-FU into non-poly(A)RNA. The incorporation of either [^{14}C]deoxyguanosine or [^3H]deoxyuridine into DNA was not inhibited by this drug regimen; however, the incorporation of [^3H]deoxyuridine into DNA was significantly elevated upon 12 and 24 hr of exposure to PALA alone. PALA and 5-FU treatment resulted in a 75% reduction in the concentration of UTP and no change in the concentration of 5-FUTP vs 5-FU treatment alone. Thus, the proportion of 5-FUTP in the total 5-FUTP + UTP pool was enhanced more than three-fold by the combination regimen. These results indicate that the synergistic effect of the combination of PALA and 5-FU on the growth of MDA cells correlates with an increased proportion of 5-FUTP in the pyrimidine nucleotide pool, and consequently with an enhanced incorporation of 5-FU into RNA, but not with inhibition of DNA synthesis.

2. The mechanism of action of adenosine, 2'-deoxyadenosine, and cordycepin (3'-deoxyadenosine) was explored in L1210 cells in vitro under conditions where their deamination was blocked by the adenosine deaminase inhibitor, dCF. Cordycepin but not adenosine or 2'-deoxyadenosine inhibited the methylation of nuclear RNA in the presence of dCF. Upon the addition of homocysteine, adenosine produced 65% inhibition of methylation of nuclear RNA, whereas 2'-deoxyadenosine was ineffective and the inhibitory effect of cordycepin was not potentiated. Under the latter conditions, RNA synthesis as measured by [^{14}C]uridine incorporation was marginally affected (30% inhibition) by adenosine plus homocysteine, but markedly inhibited by 70% by cordycepin. Cordycepin inhibited 2'-O methylation of nuclear RNA to a greater extent than base methylation, while the combination of adenosine and homocysteine inhibited these sites to equal degrees. Moreover, cordycepin inhibited >18 S nuclear RNA four times as extensively as 4 S nuclear RNA, in comparison to the equal extents of inhibition of these two classes of nuclear RNA by adenosine plus homocysteine. A positive correlation was observed between the generation of intracellular S-adenosylhomocysteine in L1210 cells and the inhibition of methylation of nuclear RNA by adenosine plus homocysteine, but not by cordycepin. These results indicate that a significant amount of S-adenosylhomocysteine can be generated in the presence of adenosine and homocysteine, presumably via S-adenosylhomocysteine hydrolase, leading to marked inhibition of methylation of nuclear RNA in mouse lymphoid leukemia cells.

3. The effects of 5-FU on the synthesis and translation in vitro of polyadenylic acid-containing polysomal RNA [poly(A)RNA] were studied in Ehrlich ascites cells incubated in vitro. Exposure of cells for 2 hr to concentrations of 5×10^{-6} - 5×10^{-4} M 5-FU did not significantly alter the size distribution of poly(A)RNA labeled with either [^3H]adenosine or [^3H]5-FU. Incorporation of

[³H]adenosine into poly(A)RNA was reduced by 28% and 57% at concentrations of 5×10^{-5} M and 5×10^{-4} M 5-FU, respectively. The latter effect correlated with increased drug substitution which ranged from approximately 0.1% to 2% at concentrations of 5×10^{-6} - 5×10^{-4} M 5-FU. Translation of 5-FU-substituted poly(A)RNA *in vitro* in either a rabbit reticulocyte lysate or wheat germ extract system did not indicate a quantitative alteration in messenger RNA activity. Autoradiography of labeled translation products did not indicate major qualitative alterations between control and 5-FU-modified poly(A)RNA. These results suggest that impairment in the synthesis of messenger RNA occurs only at high concentrations of 5-FU, but that the integrity of the translational activity of the RNA is not significantly impaired.

4. The lethal and sublethal effects of sangivamycin were studied in sarcoma 180 *in vitro* in relation to drug concentration and duration of drug exposure. Sangivamycin lethality was found to be dependent on both drug concentration and duration of drug exposure. Pronounced effects on cell survival were observed only when sangivamycin exposure was prolonged; with prolonged drug exposure, small increments in sangivamycin concentration resulted in large increases in cell killing. Log phase cells were more susceptible to the lethal effects of sangivamycin than early plateau phase cells. Measurements of incorporation of [³H]thymidine and [³H]uridine into the acid-insoluble cell fraction demonstrated inhibition of both DNA and RNA synthesis by sangivamycin which was also dependent on drug concentration and duration of drug exposure, reflecting the lethality characteristics of sangivamycin. As sangivamycin concentration was increased, DNA synthesis was inhibited more rapidly than RNA synthesis. Flow cytometry demonstrated a concentration and time dependent accumulation of cells in the late S and G₂M region of the DNA histogram. Our findings indicate that maximum lethality is obtained by prolongation of sangivamycin exposure, and suggest that pharmacokinetic studies may be important for determining regimens which provide such exposure in man.

5. The pyrrolopyrimidine, sangivamycin, and the adenosine analog, xylosyladenine, were examined for their effects on the synthesis and methylation of polysomal RNA in Ehrlich ascites tumor cells *in vitro*. The synthesis of non-polyadenylic acid (poly(A))- and poly(A)-containing RNA was inhibited 50% at concentrations of 7×10^{-6} M and 3×10^{-6} M, xylosyladenine, respectively, when adenosine deaminase was inhibited with 2'-deoxycoformycin. Sangivamycin inhibited the synthesis of non-poly(A)- and poly(A)RNA by 50% at concentrations of 5×10^{-5} M and 2×10^{-5} M, respectively. Electrophoretic separation of non-poly(A)RNA into rRNA and tRNA indicated that the inhibitory effects of both drugs were more pronounced on 28S than on 18S rRNA, and that xylosyladenine but not sangivamycin inhibited the synthesis of tRNA. Assessment of the effects of both analogs on the methylation of polysomal RNA revealed that xylosyladenine inhibited the methylation of non-poly(A)- and poly(A)RNA while sangivamycin only weakly affected the latter species of RNA. Base methylation of the affected species of RNA was inhibited slightly more than 2'-O-methylation by both drugs. These results indicate that sangivamycin is a more selective inhibitor of polysomal RNA in comparison to xylosyladenine under conditions where adenosine deaminase is not a limiting factor.

6. The adenosine analogs tubercidin, formycin and 8-azaadenosine were examined for their effects on the synthesis and methylation of nuclear RNA in L1210 cells in vitro. Total RNA and DNA synthesis was affected to the greatest extent by tubercidin ($IC_{50} = 7 \times 10^{-6}$ M) and to an insignificant degree by 8-azaadenosine and formycin; however, the effects of the latter two drugs, but not of tubercidin, were potentiated by dCF, an inhibitor of adenosine deaminase. In the presence of dCF, RNA synthesis was inhibited by 50% at 1×10^{-4} M 8-azaadenosine and by 50% at 2×10^{-4} M formycin, while DNA synthesis was inhibited less extensively. Alkaline hydrolysis of nuclear RNA labeled with [^{14}C]uridine and L-[methyl- ^3H]methionine showed preferential inhibition of base methylation in mononucleotides, but not of 2'-O-methylation in dinucleotides, for all three drugs. This differential effect persisted to varying degrees in >18S and 4S nuclear RNA separated by electrophoresis. The reduction in base methylation in 4S RNA was associated with seven of the eight methylated nucleosides in 4S RNA separated by two-dimensional thin-layer chromatography. These results indicate that tubercidin, 8-azaadenosine and formycin can preferentially inhibit the base methylation of nuclear RNA relative to its synthesis.

Proposed Course:

Detailed analyses of RNA synthesis by the procedures outlined appears to be a sensitive and discriminating means of assessing the pharmacological action of nucleoside anticancer drugs. Future studies will entail extending this biochemical framework to the study of anticancer drugs in human tumor cells in tissue culture. These investigations will allow a direct correlation of the molecular mode of action of these drugs with their effects on cell viability as determined by cloning. Studies of sangivamycin, adriamycin, 5-iminodaunorubicin, 5-FU and 5-fluorouridine are presently underway using human colon carcinoma (HT-29) cells in culture.

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Stern, H.J. and Glazer, R.I.: Inhibition of methylation of nuclear RNA in L1210 cells by tubercidine, 8-azaadenosine and formycin. Biochem. Pharmacol. 29: 1459-1464, 1980.

Ardalan, B., Cooney, D.A., Jayaram, H.N., Carrico, C.K., Glazer, R.I., Macdonald, J. and Schein, P.S.: Mechanisms of sensitivity and resistance of murine tumors to 5-fluorouracil. Cancer Res. 40: 1431-1437, 1980.

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Ardalan, B. and Glazer, R.I.: An update on the biochemistry of 5-fluorouracil. Cancer Treat. Rev. (in press), 1981.

Ardalan, B., Glazer, R.I., Kensler, T.W., Jayaram, H.N., Van Pham, T., Macdonald, J.S. and Cooney, D.A.: Synergistic effect of 5-fluorouracil and N-(phosphonacetyl)-L-aspartate on cell growth and RNA synthesis in a human mammary carcinoma. Biochem. Pharmacol. (in press), 1981.

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Glazer, R.I., Hartman, K.D. and Cohen, O.J.: The effect of sangivamycin and xylosyladenine on the synthesis and methylation of polysomal RNA in Ehrlich ascites cells in vitro. Biochem. Pharmacol. (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07111-05 LMCB								
PERIOD COVERED October 1, 1980 to September 30, 1981										
TITLE OF PROJECT (80 characters or less) Action of Anticancer Drugs on the Phosphorylation and Activity of Nonhistone Chromosomal Proteins										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: Robert I. Glazer, Ph.D.</td> <td style="width: 33%;">Pharmacologist</td> <td style="width: 15%;">LMCB</td> <td style="width: 19%;">NCI</td> </tr> <tr> <td>Other: Jeffrey D. Saffer, Ph.D.</td> <td>Research Associate</td> <td>LMCB</td> <td>NCI</td> </tr> </table>			PI: Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI	Other: Jeffrey D. Saffer, Ph.D.	Research Associate	LMCB	NCI
PI: Robert I. Glazer, Ph.D.	Pharmacologist	LMCB	NCI							
Other: Jeffrey D. Saffer, Ph.D.	Research Associate	LMCB	NCI							
COOPERATING UNITS (if any)										
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology										
SECTION Applied Pharmacology Section										
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205										
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER:								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) The <u>phosphorylation</u> of <u>nonhistone chromosomal proteins</u> (NHCP) appears to exert a <u>regulatory influence</u> on <u>transcription</u> in <u>eukaryotic cells</u>. The effects of <u>adenosine analogs</u> upon NHCP phosphorylation will be further explored by <u>analyzing the phosphorylation</u> of a discrete class of NHCP called the <u>high mobility group (HMG)</u> proteins in Ehrlich ascites cells in vitro. The association of phosphorylated HMG proteins with transcriptionally active and inactive regions in chromatin will be determined by selective nuclease digestion of nuclei.										

Objectives:

The phosphorylation of nonhistone chromosomal proteins (NHCP) is believed to exert a regulatory role on transcription in eukaryotic cells. This process is mediated via nuclear protein kinases, a heterogeneous group of enzymes. One objective of this project is to determine the effect of adenosine analogs upon the phosphorylation of a specific class of nuclear proteins known to be associated with transcriptionally active chromatin. The high mobility group (HMG) of NHCP are ideal for this study since they can be isolated relatively free of most histones and other NHCP by gel electrophoresis, and are known to be associated with the transcriptional unit of the cell, the nucleosome. In addition, little is known about the chemical modification of HMG proteins in the nucleus, and thus, investigations of their phosphorylation in transcriptionally active and inactive regions of chromatin will be carried out.

Methods Employed:

Extraction of HMG proteins with 2% trichloroacetic acid; separation and quantitation of HMG proteins by SDS and acid-urea polyacrylamide gel electrophoresis and autoradiography; labeling of NHCP and HMG proteins by incubation of L1210 or Ehrlich ascites cells in vitro with ^{32}P .

Major Findings:

1. The ability of the HMG proteins to be phosphorylated was examined in Ehrlich ascites and L1210 cells incubated in vitro. HMG proteins were selectively extracted from isolated nuclei with 2% trichloroacetic acid, and electrophoretically separated on acid-urea or SDS polyacrylamide gels. Autoradiography of the gels revealed that among the HMG proteins, only HMG 14 and 17 were labeled. The specific activities of these two proteins were approximately equal to that of histone H1. Phosphorylation of HMG 14 and 17 reached a maximum in 2-3 hr and had turnover rates in pulse-chase experiments similar to that of phosphorylated histone H1.

2. We have examined the ability of sangivamycin to inhibit the phosphorylation of the 2% trichloroacetic acid-soluble nuclear proteins from Ehrlich ascites cells in vitro. In whole cells, sangivamycin inhibited histone H1 phosphorylation with 50% inhibition observed at a drug concentration of 100 μM , but phosphorylation of the HMG proteins, HMG 14 and 17, was unaffected. At all concentrations, histone H1 phosphorylation was inhibited to a greater degree than nRNA synthesis. The pyrrolopyrimidine analogs toyocamycin, sangivamycin-amidine and sangivamycin-amidoxime did not differ substantially from sangivamycin in their inhibitory effects on histone H1 phosphorylation and nRNA synthesis in whole cells, whereas thiosangivamycin was approximately 50-fold more potent. Cell-free assays with partially purified nuclear protein kinase activities, PK-I and PK-II, revealed that sangivamycin was a competitive inhibitor vs ATP with either histone H1 or casein as substrate. The 50% inhibitory concentration of thiosangivamycin for PK-I activity was 40-fold less than sangivamycin and closely paralleled their relative inhibitory activities for inhibiting histone H1 phosphorylation in intact cells in vitro. This relationship was not apparent for sangivamycin-amidine, sangivamycin-amidoxime and toyocamycin.

3. The distribution of the phosphorylated HMG proteins in Ehrlich ascites cell chromatin has been investigated using DNase I and micrococcal nuclease. [³²P]-HMG 14 and 17 were preferentially associated with nuclease sensitive regions as demonstrated by an increase in the specific activity of these proteins in the nuclease solubilized fraction and a corresponding decrease in the nuclease insensitive fraction as compared to whole nuclei. HMG 1 and 2, which were not phosphorylated, were observed to be easily released by both nucleases. Several other 2% TCA-soluble nuclear proteins are described whose phosphorylated forms are also distributed non-randomly. These results suggest phosphorylation may control the behavior of these proteins and their role in chromatin.

Proposed Course:

The inter-relationship between the phosphorylation of HMG 14 and 17, and the phase of the cell cycle will be further investigated in synchronous populations of Chinese hamster ovary (CHO) cells and human colon carcinoma (HT-29) cells. An additional aspect to this study will entail determining the influence both in vitro and in vivo of intercalating drugs such as adriamycin on the sensitivity of [³²P]HMG 14 and 17 to release from chromatin by endonucleases such as DNase I and micrococcal nuclease.

Publications:

Saffer, J.D. and Glazer, R.I.: The phosphorylation of high mobility group proteins 14 and 17 from Ehrlich ascites and L1210 cells in vitro. Biochem. Biophys. Res. Commun. 93: 1280-1285, 1980.

Saffer, J.D. and Glazer, R.I.: Inhibition of histone H1 phosphorylation by sangivamycin and other pyrrolopyrimidine analogs. Mol. Pharmacol. (in press), 1981.

Saffer, J.D. and Glazer, R.I.: The distribution of phosphorylated HMG proteins in transcriptionally active and inactive chromatin. Nucleic Acids Res. (in press), 1981.

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Biochemistry Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1980 - September 30, 1981

Scope and General Summary of Accomplishments

The Biochemistry Section has continued to examine the mechanisms by which oncolytic analogs or antagonists of the dicarboxylic amino acids inflict damage on normal and cancerous tissues. In addition, since these oncolytic amino acids act by interrupting purine or pyrimidine biosynthesis at the steps whereat their normal counterparts are utilized rather than, for example, by impeding protein or amino acid biosynthesis, the Section has begun to examine the susceptibility of all the enzymic steps in these pathways to pharmacologic attack by agents other than the analogs of the dicarboxylic amino acids. This amplification of scope has required considerable 'methods-development' because certain of the enzymic steps involved in purine and pyrimidine biosynthesis are complex and poorly studied. Out of this effort have emerged two novel strategies for measuring L-5,6-dihydroorotic acid dehydrogenase and IMP dehydrogenase. Using the latter strategy, it has been possible to demonstrate unequivocally that one prompt and powerful consequence of the parenteral administration of the new top-priority C-nucleoside, NSC-286193D, is to inhibit the biosynthesis of XMP (and so of GMP, GDP, GTP, dGDP and DNA) most probably via inhibition of IMP dehydrogenase. Not ascertained as yet, is the exact molecular species responsible for this effect.

Staff

The permanent staff, at present, consists of two senior scientists, and a junior scientist at the GS-9 level.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07116-02 LMCB
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less) Pharmacologic Interference with the Metabolism of the Dicarboxylic Amino Acids		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT PI: Hiremagalur N. Jayaram Other: David A. Cooney Pharmacologist Head, Biochemistry Section LMCB LMCB NCI NCI		
COOPERATING UNITS (if any) City of Hope National Medical Center; Arthur D. Little Inc.		
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology		
SECTION Biochemistry Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) The mechanisms by which transplantable murine tumors become resistant to <u>Acivicin</u> , <u>DON</u> , <u>PALA</u> and <u>L-Alanosine</u> have been studied. All of these <u>amino acid antimetabolites</u> act by interfering with <u>nucleic acid biosynthesis</u> . Resistance to them is accompanied by an increase in the basal specific activity of their target enzymes and/or the enzymes of nucleic acid salvage.		

I. Studies with Amino Acid Antimetabolites

1. Acivicin: [L-(α S,5S)- α -amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid; NSC-163501; AT-125] was isolated from the fermentation broths of Streptomyces svaceus and found to exhibit significant antitumor activity against LT210 and P388 leukemia in mice, as well as against certain human tumor xenografts in athymic mice. Recently this drug has been evaluated in Phase I and Phase II trials in man.

In last year's annual report we recapitulated our biochemical and pharmacologic studies with this antibiotic versus P388 tumors sensitive (P388/S) and resistant (P388/ACIA) to this drug.

Collateral sensitivity to PALA of a P388 tumor (P388/ACIA) rendered resistant to Acivicin: Administration of PALA [N-(phosphonacetyl)-L-aspartic acid], NSC-224131, is ineffective in treating mice bearing the parent P388 leukemic line; however, such treatment becomes highly effective when the parent P388/S leukemia is rendered resistant to Acivicin (P388/ACIA). This phenomenon of collateral sensitivity is associated with a significantly higher inhibition of the specific activities of the inaugurating enzyme of the de novo pyrimidine biosynthesis pathway, carbamyl phosphate synthetase II, and of the purine and pyrimidine salvage pathways in the PALA-sensitive line, P388/ACIA. Twenty-four hours following the administration of PALA, 200 mg/kg i.p. to tumor-bearing mice, the intracellular concentration of the pyrimidine nucleoside triphosphates were decreased in the P388/ACIA, PALA-sensitive cells, whereas, no significant change in the corresponding nucleotide pool sizes were observed in P388/S, the PALA-resistant line. Moreover, the concentration of purine nucleotides underwent a significant expansion only in the P388/ACIA line following a similar treatment with PALA. It is proposed that the imbalance in the generation of pyrimidine and purine nucleotides may underlie the observed collateral sensitivity to PALA of the P388/ACIA leukemia line.

2. Studies on the mechanism of action of DON [6-diazo-5-oxo-L-norleucine, NSC-7365]: DON is an antagonist of L-glutamine with notable antitumor activity against several murine leukemias. Interest in DON renewed as a consequence of the finding of its effectiveness in treating human lung and colon xenografts in athymic mice. We have used the native DON-sensitive P388 leukemia as a model to study the mechanism of action of DON. [6- ^{14} C]DON was transported by P388 cells with a K_m of 0.66 mM and a V_{max} of 0.33 nmoles of the drug transported/million cells/min, i.e., at a rate about 20 times slower than L-glutamine. L-Glutamine competitively inhibited this transport. Four hours following the intraperitoneal administration of DON (20 mg/kg) to tumor bearing mice, PRPP amidotransferase, GMP synthetase, fructose-6-phosphate amidotransferase, L-asparagine synthetase and L-glutaminase activities were potently inhibited (>50%), whereas the specific activity of carbamyl-phosphate synthetase was only marginally repressed; dialysis against buffer of the extracts prepared from treated tumors failed to reverse inhibition of the enzyme activities. Maximum inhibition of susceptible L-glutamine amidotransferases was observed within 4-8 hours of the administration of DON; complete restitution of the enzyme activities was achieved in 48 hours. Analysis of acid-soluble nucleotide pools after treatment with DON indicated a reduction in the concentration both of adenine

and guanine nucleotides. Furthermore, the synthesis of RNA and DNA by cells in culture was potently inhibited, whereas protein synthesis was not affected. A study of the ability of purines, pyrimidines and amino acids to reverse the cytotoxicity of DON to P388 cells revealed that adenine, adenosine, hypoxanthine and L-glutamine were effective counteragents. These studies confirm that DON behaves principally as an inhibitor of the de novo purine biosynthetic pathway.

3. Studies with PALA:

a. Alterations in PRPP and Uridine Nucleotide Pools in Lewis Lung Carcinoma Produced by PALA. A variant of the Lewis Lung Carcinoma (LL) resistant to PALA was developed by exposure to sub-therapeutic doses of the drug. This variant showed markedly elevated activities of the first 3 enzymes of pyrimidine biosynthesis [e.g. carbamyl phosphate synthetase II (CPS II) + 3x]. Inasmuch as mammalian CPS II is subject to endogenous regulation, measurements were made of these effectors in the PALA-sensitive and resistant tumors. The net concentrations of uridine nucleotides (Σ U) (500 μ M) and of PRPP (55 μ M) were elevated 2-fold in the PALA-resistant variant when compared to the parent line (285 and 29 μ M). Since these intermediary metabolites exert opposite effects on CPS II (PRPP(+), Σ U(-)), the unidirectional changes in them probably tend to cancel out, leaving the enzyme functioning normally. To determine the enzymologic basis for the elevated levels of these effectors, the systems principally responsible for the generation and consumption of UTP and PRPP were measured. Enhanced de novo pyrimidine biosynthetic activity in the resistant variant may explain the augmented pool of Σ U. Treatment of mice bearing sensitive or resistant LL with PALA (400 mg/kg) provoked sharp decreases (70-80%) in Σ U and PRPP. The decrease in Σ U is due to inhibition of the de novo pathway. PALA also provokes elevations in purine nucleotide pools (50-100%) which may reflect increased utilization of PRPP and thus diminished levels. However, PRPP synthetase, PRPP amidotransferase, orotate phosphoribosyl transferase, PRPP phosphohydrolase and HGPRTase were present at equivalent specific activities in both lines, irrespective of whether PALA or saline was administered. Thus, the perturbations in PRPP and Σ U levels produced by PALA can be explained, but the steady-state change in PRPP due to resistance cannot.

b. Measurements of the Specific Activities of All Six Enzymes which Participate in the de novo Pyrimidine Biosynthetic Pathway. During the present year, the Section devoted considerable energy to the preparation of a tabulation and characterization of as many as possible of the known inhibitors of the de novo pyrimidine biosynthetic pathway. These data have been brought together in a book-chapter, undergoing publication in the Advances in Pharmacology and Chemotherapy Series from Academic Press. In the course of this endeavor, it became clear that the most rational strategy for the deployment of these inhibitors, as drugs, required a firm knowledge of the specific activities of their target enzymes in the panel of tumors used routinely for screening by the National Cancer Institute. It was also desired to determine whether the specific activities of the constituent enzymes of the two multi-enzyme complexes which participate in the path (CPS-ATCase-DHOase) and (OPRTase-ODCase) must vary in a coordinate way, or whether, in one or another of the tumors, an aberrancy might be detected. To answer these needs, the methodology for assessing the

specific activities of the six de novo pyrimidine enzymes was perfected and measurements made in the ten most widely used rodent tumors. The results of these determinations are presented here (Table 1). Also included, for purposes of completeness are the principal enzymes of purine and pyrimidine salvage (Table 2).

Table 1

Specific Activities of de novo Pyrimidine Biosynthetic Enzymes
in Commonly Used Transplantable Murine Tumors

Tumor Line	Specific Activity (nmol/mg/hr)			
	pyr-1 CPS II	pyr-2 ATCase	pyr-3 DHOase	pyr 1-3 Ratio
L1210 Leukemia	4.2 ± 0.3*	479 ± 37	67 ± 5	1:114:16
P388 Leukemia	5.9 ± 0.5	715 ± 58	205 ± 28	1:121:35
L5178Y Leukemia	2.0 ± 0.2	596 ± 47	123 ± 10	1:298:62
Ehrlich Ascites Carcinoma	2.0 ± 0.6	634 ± 55	72 ± 6	1:317:36
P815 Mastocytoma	4.1 ± 0.3	841 ± 49	65 ± 10	1:205:16
ADJ/PC6	0.6 ± 0.1	691 ± 59	149 ± 15	1:1152:248
Lewis Lung Carcinoma	1.0 ± 0.1	127 ± 10	22 ± 4	1:127:22
B16 Melanoma	2.1 ± 0.1	373 ± 26	63 ± 4	1:178:30
Colon Carcinoma 26	2.3 ± 0.2	249 ± 18	116 ± 14	1:108:50
Colon Carcinoma 38	0.3 ± 0.1	366 ± 19	250 ± 13	1:1220:833
M5076 Ovarian Carcinoma	5.3 ± 0.5	398 ± 17	190 ± 10	1:75:36
Glioma 26	2.5 ± 0.5	580 ± 47	108 ± 11	1:232:43
	pyr-4 DHO deHase	pyr-5 OPRTase	pyr-6 OMP deCase	pyr 5,6 Ratio
L1210 Leukemia	28.6 ± 1.3	4.9 ± 0.4	15.0 ± 1.1	1:3.1
P388 Leukemia	13.5 ± 1.8	11.6 ± 1.2	27.1 ± 2.8	1:2.3
L5178Y Leukemia	43.6 ± 3.3	28.1 ± 2.2	93.0 ± 11	1:3.3
Ehrlich Ascites Carcinoma	8.6 ± 1.3	6.4 ± 0.6	24.1 ± 2.0	1:3.8
P815 Mastocytoma	26.5 ± 0.9	20.2 ± 1.0	46.6 ± 1.7	1:2.3
ADJ/PC6	22.2 ± 1.2	20.8 ± 0.9	47.2 ± 3.0	1:2.3
Lewis Lung Carcinoma	8.4 ± 0.7	4.3 ± 0.5	14.0 ± 0.8	1:3.3
B16 Melanoma	20.7 ± 3.1	6.3 ± 0.5	27.9 ± 1.9	1:4.4
Colon Carcinoma 26	8.6 ± 0.9	30.6 ± 2.9	86.5 ± 6.7	1:2.8
Colon Carcinoma 38	8.4 ± 0.6	4.6 ± 0.2	10.6 ± 0.4	1:2.3
M5076 Ovarian Carcinoma	6.4 ± 1.0	3.9 ± 0.4	15.5 ± 1.7	1:4.0
Glioma 26	46.2 ± 2.8	24.8 ± 3.0	102.8 ± 7.5	1:4.1

*Mean ± S.E. (n = 5)

Table 2

Specific Activities in the Most Commonly Used Murine Transplantable Tumors
of the Enzymes of Purine and Pyrimidine Salvage

Tumor System	Specific Activity (nmol/hr/mg protein); mean $\pm$ S.E.; n = 5				
	APRT-ase	HPRT-ase	Cytidine Nucleoside Kinase	Uridine Nucleoside Kinase	Deoxy- cytidine Nucleoside Kinase
P388 Leukemia	186.9 $\pm$ 1.8	214.0 $\pm$ 6.7	158.1 $\pm$ 14.4	167.0 $\pm$ 24.3	27.5 $\pm$ 4.4
L5178Y Leukemia	147.8 $\pm$ 10.4	273.3 $\pm$ 11.2	229.7 $\pm$ 28.7	172.4 $\pm$ 6.3	6.2 $\pm$ 0.2
Colon Carcinoma 26	274.5 $\pm$ 52.7	91.8 $\pm$ 12.6	485.9 $\pm$ 12.1	199.8 $\pm$ 8.3	5.6 $\pm$ 0.3
Colon Carcinoma 38	335.4 $\pm$ 24.3	265.4 $\pm$ 12.2	282.9 $\pm$ 16.9	305.4 $\pm$ 20.2	20.6 $\pm$ 2.5
Ehrlich Ascites Carcinoma	59.8 $\pm$ 2.7	91.2 $\pm$ 9.8	51.8 $\pm$ 11.5	67.8 $\pm$ 1.0	2.7 $\pm$ 0.8
P815 Mastocytoma	95.9 $\pm$ 6.4	190.6 $\pm$ 8.8	527.5 $\pm$ 24.9	284.4 $\pm$ 27.8	12.7 $\pm$ 2.1
Plasma Cell Cytoma (ADJ/PC-6)	112.6 $\pm$ 13.5	235.9 $\pm$ 14.3	293.4 $\pm$ 11.3	441.6 $\pm$ 15.0	6.6 $\pm$ 0.5
Lewis Lung Carcinoma	250.7 $\pm$ 11.7	137.1 $\pm$ 2.1	178.0 $\pm$ 27.4	135.6 $\pm$ 18.8	3.2 $\pm$ 0.2
B-16 Melanoma	50.8 $\pm$ 6.4	62.1 $\pm$ 4.7	76.4 $\pm$ 12.5	79.2 $\pm$ 18.1	1.2 $\pm$ 0.3
M5076 Ovarian Teratocarcinoma	235.0 $\pm$ 11.7	334.8 $\pm$ 13.5	34.5 $\pm$ 4.0	39.8 $\pm$ 3.3	7.1 $\pm$ 1.2
Glioma 26	194.7 $\pm$ 8.2	273.0 $\pm$ 15.3	112.2 $\pm$ 12.1	126.2 $\pm$ 25.0	28.5 $\pm$ 3.2

4. Studies with L-Alanosine

In last year's annual report, it was documented that, in tumors rendered resistant to L-Alanosine, the concentration of the active anabolite of the drug, 'L-Alanosyl AICOR', was significantly lower than in their counterparts. These studies have now been extended to the panel of widely-used transplantable rodent tumors discussed above. For purposes of completeness, the specific activities of the enzymes participating in the metabolism of L-Alanosine were determined as was the chemotherapeutic activity of the antibiotic. These results are presented in Table 3. It can be seen, in general, that tumors rich in SAICAR kinosynthase, accumulated higher concentrations of 'L-Alanosyl AICOR' and tended to be sensitive to the drug. Thus, the parameters which, in artificially resistant tumors, correlated with chemotherapeutic sensitivity to L-Alanosine, also appear to explain sensitivity or resistance in the naturally sensitive or resistant case.

Table 3

The Concentration of L-Alanosyl-AICOR and of L-Alanosine-Metabolizing Enzymes in Tumors Sensitive and Resistant to the Drug

Tumor	Specific Activity (nmoles/hr/mg protein $\pm$ S.D.)				Sensitivity to L-Alanosine
	Adenylo-succinate Synthetase	Adenylo-succinate Lyase	SAICAR Synthetase	Ala-AICOR [μ M]	
Lewis Lung Carcinoma	6.70 $\pm$ 0.88	340 $\pm$ 76	2.66 $\pm$ 0.36	68.0	R
P388 Leukemia	16.14 $\pm$ 2.05	273 $\pm$ 19	10.11 $\pm$ 1.20	83.7	S
B-16 Melanoma	17.41 $\pm$ 9.18	350 $\pm$ 64	4.77 $\pm$ 2.50	NOT DONE	R
Colon Carcinoma 38	5.00 $\pm$ 1.90	418 $\pm$ 65	1.34 $\pm$ 0.34	56.3	R
Ovarina Tereto-carcinoma	17.8 $\pm$ 1.24	379 $\pm$ 86	10.3 $\pm$ 4.0	16.6	R
Ehrlich Ascites*	8.63 $\pm$ 0.57	291 $\pm$ 45	2.10 $\pm$ 0.29	6.6	S
Glioma-26	8.63 $\pm$ 0.57	1038 $\pm$ 286	23.5 $\pm$ 6.8	7.5	S
L5178Y Leukemia	9.16 $\pm$ 4.11	185 $\pm$ 36	5.36 $\pm$ 0.90	120	S
Colon Carcinoma 26	12.54 $\pm$ 1.91	715 $\pm$ 324	14.47 $\pm$ 0.50	108	R
P-815 Mastocytoma	5.61 $\pm$ 0.91	663 $\pm$ 103	4.56 $\pm$ 1.20	NOT DONE	NOT DONE
L1210 Leukemia	7.90 $\pm$ 1.94	321 $\pm$ 30	6.58 $\pm$ 1.27	89.8	S

*Subcutaneous; this tumor does not thrive in the subcutis.
Days 1-10 ip, dose range 8-100 mg/kg

Publications

Ardalan, B., Kensler, T.W., Jayaram, H.N., Morrison, B., Chote, D., Chadwick, M., Liss, R., Cooney, D.A.: Longterm association of PALA with bone. Cancer Res. 41: 150-156, 1980.

Kensler, T.W., Erlichman, C., Jayaram, H.N., Tyagi, A.K., Ardalan, B. and Cooney, D.A.: Peripheral leucocytes as indicators of the enzymic effects of N-(phosphonacetyl)-L-aspartic acid on human L-aspartate transcarbamylase activity. Cancer Treat. Rep. 64: 967-973, 1980.

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Kensler, T.W., Reck, L.J. and Cooney, D.A.: Therapeutic effects of acivicin and N-(phosphonacetyl)-L-aspartic acid in a biochemically designed trial against a N-(phosphonacetyl)-L-aspartic acid-resistant variant of the Lewis lung carcinoma. Cancer Res. 41: 905-909, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07122-01 LMCB
PERIOD COVERED October 1, 1980 to September 30, 1981		
TITLE OF PROJECT (80 characters or less) Studies with Nucleosides and Bases		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT Co-PI's: David A. Cooney Hiremagalur N. Jayaram Head, Biochemistry Section Pharmacologist LMCB NCI LMCB NCI		
COOPERATING UNITS (if any) NCI/VA (D. Carney); Lombardi Cancer Center (P. Schein)		
LAB/BRANCH Laboratory of Medicinal Chemistry		
SECTION Biochemistry Section		
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS		
SUMMARY OF WORK (200 words or less - underline keywords) Both <u>in vitro</u> and <u>in vivo</u> exposure of susceptible tumor-cells to NSC 286193D results in an inhibition of the conversion of IMP to GMP. The state of <u>guanine nucleotide</u> starvation so produced arrests the synthesis of DNA and RNA and leads to the death of P388 and Lewis lung cells. Although the locus of action of this nucleoside appears to be at the level of <u>IMP dehydrogenase</u> , there was no correlation between the specific activity of this enzyme and the susceptibility of murine or human tumor cells to cytotoxicity from the drug.		

A. Studies with Nucleosides and Bases

1. Studies with NSC 286193D: In 1976, Srivastava and Robins described the synthesis of a series of C-glycosyl nucleosides structurally related to the antiviral drug ribavirin. One of these, NSC 286193D, a thiazole, exhibited significant antiviral activity against Type I herpes simplex virus, Type 3 parainfluenza virus as well as Type 13 rhinovirus; this compound, like ribavirin, also interrupted the incorporation of radio-labeled hypoxanthine into the guanine, but not adenine nucleotides of Ehrlich Ascites tumor cells. Pursuant to these findings, a systematic examination of the oncolytic properties of the novel thiazole nucleoside was undertaken at the National Cancer Institute: these studies warranted the conclusion that the agent exhibited notable antineoplastic activity against several murine leukemias and at least one solid tumor, the intravenous Lewis Lung Carcinoma, against which it was, under certain circumstances, curative. This latter activity is virtually unprecedented. The present biochemical pharmacologic studies were undertaken in order to gain further insight into the mechanism of action of this new oncolytic drug. For these studies, P388 cells growing in vivo and in vitro were used. The compound significantly extends the lifespan of BDF₁ mice inoculated with this leukemia over a span of doses ranging from 25 to 750 mg/kg. In culture, the proliferation of P388 cells was reduced by 50% at a concentration of the nucleoside of $\sim 1 \mu\text{M}$.

Upon exposure to concentrations of the thiazole nucleoside which were antiproliferative, the synthesis of RNA and DNA were halted in parallel whereas protein synthesis was little affected. The macromolecular incorporation of all the common pyrimidine precursors was inhibited by the drug, but, among the purines this effect extended only to members of the adenine family, and in fact, the utilization of guanine and its congeners was reproducibly stimulated.

When an examination was made of the ability of a comprehensive series of preformed purines and pyrimidines to overcome the inhibition of thymidine incorporation provoked by exposure to the thiazole nucleoside, the guanines were notably effective, but xanthosine also was shown to be an active antidote. Confirmation that the drug was producing a state of guanine deprivation was provided by HPLC analogs of acid-soluble extracts of P388 cells grown in culture: a time-dependent fall in the concentration of GTP ensued upon exposure to $10 \mu\text{M}$ thiazole; ATP was unaffected and UTP increased.

Pursuant to these findings, an examination was made of the enzymologic steps unique to guanine biosynthesis in cells exposed to cytotoxic concentrations of the drug. No inhibition of GMP synthetase (XMP aminase) could be demonstrated either in vivo or in vitro, but the specific activity of IMP dehydrogenase underwent substantial reductions in both of these cases. A preliminary kinetic analysis of this interaction revealed that the thiazole nucleoside inhibited a partially purified preparation of IMP dehydrogenase from P388 cells uncompetitively with IMP, and exhibited a K_i of $\sim 100 \mu\text{M}$. Since concentrations equal to or greater than this value are reached and maintained in subcutaneous nodules of this tumor following therapeutic doses of the drug, it is possible that the unchanged drug plays a role in its activity. However, by analogy to other oncolytic nucleosides, it is additionally likely that anabolism to the nucleotide occurs and that this molecule participates in the mechanism of action of the drug.

The foregoing results suggested that measurements of IMP dehydrogenase in extracts of tumors scheduled to receive NSC 286193D might be of predictive value. To test this point, the specific activity of the enzyme was measured in P388 cells sensitive and (rendered) resistant to the agent, as well as in a panel of human lung tumor lines growing in culture. As Table 4 illustrates, no strict correlation between susceptibility to the thiazole nucleoside, and the apparent activity of IMP dehydrogenase, was demonstrable.

Table 4
Sensitivity to NSC 286193D and Purine Enzymology in Human
Lung Cancer Cells Grown in Culture

Human Cell lines	Sensitivity to NSC 286193D (ID ₅₀)	ENZYME ACTIVITIES				
		(nmoles/mg protein/hr)				
		APRTase	HPRTase	Adenosine- kinase	IMP- dehydrogenase	XMP- aminase
#1 NCIH60	0	7.4	42.3	1.4	39.8	0.9
#2 NCIH69	0	13.0	73.8	5.1	14.7	4.8
#3 NCIH146	0	15.6	103.7	6.9	35.2	4.4
#4 NCIH187	10 ⁻⁶ M	9.9	43.4	1.6	19.6	<.1
#5 NCIH209	10 ⁻⁴ M	12.7	32.1	6.4	12.2	1.4
#6 NCIH82	10 ⁻⁶ M	20.8	67.1	10.7	114.4	1.7
#7 NCIN417	0	14.0	92.6	3.6	64.6	0.8
#8 NCIH23	0	33.3	58.5	8.8	38.2	1.9
#9 NCIH234	NT	10.3	10.6	3.2	34.7	2.9
#10 NCIH249	0	9.0	60.1	3.6	15.7	3.6
P388 line	10 ⁻⁶ M	-	-	-	5.1	-
P388/Resistant line	10 ⁻² M	-	-	-	9.6	-

2. Studies with 5-FU: In collaboration with Dr. Philip Schein of the Lombardi Center at Georgetown University Hospital, studies are being undertaken to assess the metabolism of [^{14}C]5-FU by clones or colonies of human colon cancers grown in vitro according to the methodology of Salmon/Hamburger. These investigations are intended to be predictive of responsiveness, and will ultimately be correlated with clinical response. The paucity of cellular material and the need for simplicity together have dictated a streamlined approach.

As soon as is feasible after the establishment of colonies, [^{14}C]5-FU will be added to satellite cultures, at pharmacologically meaningful concentrations. Parallel cultures will receive equimolar 5-FU, followed in 2 h by 5 μCi of [$5\text{-}^3\text{H}$]UdR. In the former case, acid soluble and acid insoluble fractions will be prepared and the disposition of [^{14}C] in each determined. In the latter case, tritiated water accruing from the conversion of [$5\text{-}^3\text{H}$]dUMP to TMP will be distilled by the microdistillation technique worked out in this laboratory and described in last year's annual report. It has been found that the de-tritiation of [$5\text{-}^3\text{H}$]UdR by as few as 1×10^5 cells can be detected with this approach.

Publications:

Ardalan, B., Macdonald, J.S., Cooney, D.A., Koppman, M. and Schein, P.: Prediction of clinical response to 5-fluorouracil containing chemotherapy; preliminary results of in vitro assay in human breast cancer. In Mathe, G. and Muggia, F. (Eds.): Recent Results in Cancer Research. Berlin, Springer-Verlag, 1980, pp. 84-90.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07123-01 LMCB																
PERIOD COVERED October 1, 1980 to September 30, 1981																		
TITLE OF PROJECT (80 characters or less) Toxicologic Studies of Interferon in Mice																		
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 40%;">PI: David A. Cooney</td> <td style="width: 30%;">Head, Biochemistry Section</td> <td style="width: 15%;">LMCB</td> <td style="width: 15%;">NCI</td> </tr> <tr> <td>Other: Hiremagalur N. Jayaram</td> <td>Pharmacologist</td> <td>LMCB</td> <td>NCI</td> </tr> <tr> <td>Marion Copley</td> <td>Pathologist</td> <td>LCHP</td> <td>NCI</td> </tr> <tr> <td>Yvonne Wilson</td> <td>Biologist</td> <td>LMCB</td> <td>NCI</td> </tr> </table>			PI: David A. Cooney	Head, Biochemistry Section	LMCB	NCI	Other: Hiremagalur N. Jayaram	Pharmacologist	LMCB	NCI	Marion Copley	Pathologist	LCHP	NCI	Yvonne Wilson	Biologist	LMCB	NCI
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COOPERATING UNITS (if any) Laboratory of Chemical Pharmacology, NCI																		
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology																		
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CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (a1) MINORS ☐ (a2) INTERVIEWS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER																		
SUMMARY OF WORK (200 words or less - underline keywords) Three batches of Interferon were administered intraperitoneally to mice at doses up to 20×10^6 I.U./kg. In all cases, the preparations were well tolerated as adjudged by weight-status, hemograms and the absence of pathology.																		

Toxicologic Studies with Interferon in Mice

The three batches of Interferon used in these studies were provided by Dr. John Douros of the National Cancer Institute. Immediately prior to use, the product was dissolved in sterile saline. Male CDF₁ mice were procured by Mrs. Ruth Davis of the NCI. Mice weighing about 21 g were selected for the experiment. Groups of four mice were individually marked and given intraperitoneal injections of 0.5 ml each of saline or Interferon ($\sim 20 \times 10^6$ I.U./kg). Water and Purina Rat and Mouse ration were supplied to the mice ad libitum. The mice were weighed daily and examined for any gross changes. The animals were sacrificed 8 days after injection and the following tissues were taken for histopathologic examination: spleen, liver, mesenteric lymph node, submandibular lymph node, femoral bone marrow, thymus, diaphragm, peritoneal wall, heart, lung, trachea, salivary gland, esophagus, stomach, duodenum, ileum, kidney, pancreas, adrenal gland, thyroid gland, parathyroid gland, testis and gall bladder. Blood for WBC and RBC was taken in heparinized tubes at the time of sacrifice. Tissues examined grossly included all abdominal and thoracic organs. Other organs examined included thyroids, salivary glands, submandibular lymph node, accessory sex organs, gonads, skeletal muscle, and brain. No significant weight loss was detected during the 8 day observation period at the dose given. Leucocyte and erythrocyte counts were not changed by the treatment. There were no treatment-related lesions observed at necropsy in either of the two groups. Several mice did, however, exhibit white streaks on the epicardium in both treated and control groups. There were no treatment-related lesions observed in the tissues examined using a routine hematoxylin-eosin stain on paraffin sections. There were several spontaneous lesions in both treated and control animals. These included: mesenteric lymph node - lymphangiectasis (minimal, 1 animal); heart - epicardial mineralization (slight-moderate, 5 animals); heart - focal chronic myocarditis (minimal, 1 animal); trachea - dilated and hyperplastic glands (minimal-moderate, 6 animals). In summary, the 3 batches of Interferon used in this study were generally well tolerated by mice at the doses given.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07124-01 LMCB								
PERIOD COVERED October 1, 1980 to September 30, 1981										
TITLE OF PROJECT (80 characters or less) Methods Development										
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT <table style="width: 100%; border: none;"> <tr> <td style="width: 33%;">PI: David A. Cooney</td> <td style="width: 33%;">Head, Biochemistry Section</td> <td style="width: 15%;">LMCB</td> <td style="width: 19%;">NCI</td> </tr> <tr> <td>Other: Hiremagalur Jayaram</td> <td>Pharmacologist</td> <td>LMCB</td> <td>NCI</td> </tr> </table>			PI: David A. Cooney	Head, Biochemistry Section	LMCB	NCI	Other: Hiremagalur Jayaram	Pharmacologist	LMCB	NCI
PI: David A. Cooney	Head, Biochemistry Section	LMCB	NCI							
Other: Hiremagalur Jayaram	Pharmacologist	LMCB	NCI							
COOPERATING UNITS (if any)										
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology										
SECTION Biochemistry Seciton										
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205										
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:								
CHECK APPROPRIATE BOX(ES) ☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER ☐ (a1) MINORS ☐ (a2) INTERVIEWS										
SUMMARY OF WORK (200 words or less - underline keywords) Facile <u>tritium-release assays</u> for IMP and <u>dihydroorotate dehydrogenase</u> have been designed, implemented and validated. Both trap the tritiated water released in the course of enzymic dehydrogenation within droplets of concentrated alkali following quantitative microdistillation.										

Methods Development

A. Development of a Tritium-Release Assay for Inosinic Acid Dehydrogenase

G[³H]Inosinic acid (G[³H]IMP) has been biosynthesized in good yield from G[³H]-hypoxanthine via the action of a partially purified preparation of hypoxanthine/guanine phosphoribosyl transferase from mouse brain. The product was purified in one step by ascending paper chromatography, and used to assess the activity of IMP dehydrogenase in extracts of a variety of organs, organelles, and organisms. To conduct the assay, tritiated substrate is admixed with enzyme in a final volume of 10 μ l; NAD is present to serve as cofactor for the reaction, uridine to retard the phosphorolysis of any inosine formed (by phosphatase decomposition of IMP) and allopurinol to inhibit the oxidation of any hypoxanthine generated. After an appropriate period of incubation, any NAD[³H] formed is allowed to decompose spontaneously, and the resulting [³H]₂O is isolated by quantitative microdistillation. The assay, performed as described, is facile, sensitive and accurate, with the capability of detecting the decomposition of as little as 200 pmoles of [³H]IMP. Using it, measurements have been made of IMP dehydrogenase in a variety of organs, organelles and organisms. The major finding to emerge from this survey is that pancreas contains the enzyme at the highest specific activity of any organ examined. This is, of course, consistent with the vigorous rates of macromolecular synthesis carried out by this gland.

B. Development of a Tritium-Release Assay for L-5,6-Dihydroorotate Dehydrogenase.

In a strategy analogous to that given above, [³H]L-5,6-dihydroorotic acid has been synthesized enzymatically from [³H]L-aspartic acid, in 40% yield. Following purification and adjustment of the specific activity of the product, it has been used to assess DHO dehydrogenase under a variety of conditions in a variety of organs. Microdistillation of the [³H]₂O arising from this dehydrogenation was conducted as described in the preceding section. Activity was linear with time and protein, and could be totally inhibited by dichloroallyl lawsone. Only mitochondria and, to a minor extent, nuclear membrane attacked the substrate. No exogenous cofactors were required. Measurements of the specific activity of DHO dehydrogenase made by the tritium release assay are arrayed in Table 1, supra.

Publications:

Park, K.W., Tyagi, A.K. and Cooney, D.A.: A radiometric technique for the measurement of adenylosuccinate lyase. J. Biochem. Biophys. Methods 2: 291-297, 1980.

Jayaram, H.N., Kensler, T.W. and Ardalan, B.: A radiometric enzyme-inhibition technique for measuring acivicin (L-[α S,5S]- α -amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid, NSC-163501) in plasma and tissues. Cancer Treat. Rep. (in press), 1981.

Kensler, T.W., Han, N. and Cooney, D.A. A straightforward method for the simultaneous preparation of radiolabeled L-dihydroorotic and N-carbamyl-L-aspartic acids. Anal. Biochem. (in press), 1981.

Molecular Biology and Methods Development Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1980 - September 30, 1981

The Section of Molecular Biology and Methods Development is concerned with the development of regimens for the potentiation of cancer chemotherapeutic activities. Its staff consists of the Section Head, two Senior Staff Fellows, one Visiting Fellow, two senior technicians, and two part time college students. A major effort of our Section has been concerned with the biochemical basis of melphalan resistance in L1210 tumor cells. Further evidence for the role of glutathione in resistance has been achieved and a plasma membrane sulfhydryl group has been implicated.

Nutritional and pharmacological regimens which may determine therapeutic potency are explored in both tissue culture and the tumor-bearing animal in attempts to direct cytotoxic agents toward the tumor cell and away from sensitive normal tissues. Syntheses of new cytotoxic and potentiating agents are undertaken to achieve this goal. Biochemical studies have included analysis of inhibition kinetics of binding, transport and detoxification of cytotoxic agents in sensitive and resistant tumor cells and sensitive host tissues. Results are described from analysis of data on the function of the nucleoside and amino acid transport systems in both L1210 and bone marrow progenitor cells.

The life-threatening host toxicity of nitrogen mustards has led us to propose for evaluation a series of less toxic nitrogen mustard analogs with arms of unequal reactivity. These contain a β -chloroethyl and a γ -chloropropyl on either the same or different nitrogen atoms. One new compound in this category, 1-(2-chloroethyl)-4-(3-chloropropyl)piperazine, shows moderate activity in the L1210 system (60% ILS) and at an optimal schedule gives comparable results with ordinary nitrogen mustards. It has been proposed for evaluation in a tumor spectrum. Corresponding analogs of aniline mustard, phenylacetic acid mustard and phenylalanine mustard are in preparation for a more complete study.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 03579-08 LMCB																				
PERIOD COVERED October 1, 1980 to September 30, 1981																						
TITLE OF PROJECT (80 characters or less) Effect of Chemotherapeutic Agents on L1210 Cell Cycle Progression																						
NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT																						
<table style="width: 100%; border: none;"> <tr> <td style="width: 15%;">PI:</td> <td style="width: 45%;">Robert L. Dion</td> <td style="width: 20%;">Chemist</td> <td style="width: 10%;">LMCB</td> <td style="width: 10%;">NCI</td> </tr> <tr> <td>Other:</td> <td>Vincent H. Bono, Jr., M.D.</td> <td>Chief, IDB</td> <td>LMCB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Marco Rabinovitz, Ph.D.</td> <td>Head, MBMDS</td> <td>LMCB</td> <td>NCI</td> </tr> <tr> <td></td> <td>Maurice Stauquet, M.D.</td> <td>Visiting Scientist, IDB</td> <td>CTEP</td> <td>NCI</td> </tr> </table>			PI:	Robert L. Dion	Chemist	LMCB	NCI	Other:	Vincent H. Bono, Jr., M.D.	Chief, IDB	LMCB	NCI		Marco Rabinovitz, Ph.D.	Head, MBMDS	LMCB	NCI		Maurice Stauquet, M.D.	Visiting Scientist, IDB	CTEP	NCI
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	Maurice Stauquet, M.D.	Visiting Scientist, IDB	CTEP	NCI																		
COOPERATING UNITS (if any) The Upjohn Company, Kalamazoo, Michigan																						
LAB/BRANCH Laboratory of Medicinal Chemistry and Biology																						
SECTION Molecular Biology and Methods Development Section																						
INSTITUTE AND LOCATION NCI, NIH, Bethesda, Maryland 20205																						
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☐ (a1) MINORS ☐ (a2) INTERVIEWS																						
SUMMARY OF WORK (200 words or less - underline keywords)																						
Project is designed to investigate the effects of <u>cancer chemotherapeutic agents on cell cycle progression</u> based on their cytotoxicity in an in vitro L1210 murine leukemia system. <u>DNA content distribution (DCD)</u> curves are generated with the use of a <u>flow microfluorometer</u> and drug treated curves are compared with steady state DCD curves after analysis by computer. An attempt to classify these agents as to their mode of action (<u>antimetabolite</u>, etc.) by computer analysis is in progress.																						

Project Description:

This project is concerned with development of techniques for the analysis of drug effects on the progress of cells through the proliferative cell cycle and their application of (a) the characterization of therapeutically active drugs and (b) the identification and possible action of agents with potential therapeutic activity. The major methods employed are (a) mammalian cell culture (L1210 murine leukemia in suspension), (b) flow microfluorometric (FMF) determination of cell DNA content using hypotonic citrate/propidium iodide stained nuclei in a Cytofluorograf (Bio/Physics System; Model 4801) interfaced with a multichannel pulse height analyzer and (c) processing, analyzing and plotting cell DNA content distribution (DCD) curves by computer.

During the current year the following drugs were evaluated: streptonigrin (NSC-45383), azapicyl (NSC-68626), diglycoaldehyde (NSC-118994), ftorafur (NSC-148958) and pyrazofurin (NSC-143095).

Major Findings:

NSC	Name	ID ₅₀ (μ g/ml)	DCD Effects
45383	Streptonigrin	0.28	*
68626	Azapicyl	360	*
118994	Diglycoaldehyde	31	*
143095	Pyrazofurin	< 1	*
148958	Ftorafur	14	*

*Have not been evaluated.

We have also studied the predictability of positive animal tumor screens and the correlation of these results with results obtained at the clinical level. Eight animal tumor screens and two human tumors (oat cell/lung and breast) were correlated using 24 drugs in the oat cell system and 58 drugs in the breast system. The predictive value of a positive screen (PV+) is the ratio between the number of drugs active in the screen and in the clinic over the total number of drugs positive in the screen. In order to compute the (PV+) values, it was necessary to establish a data base containing all the screening data and clinical results at the DN2 level. It was then necessary to develop the software using the BRIGHT system on the DEC-10 computer to calculate all possible screening combinations with their resultant PV+.

The mean value for breast was 0.46 ± 0.128 (2σ).

The mean value for lung was 0.58 ± 0.62 (2σ).

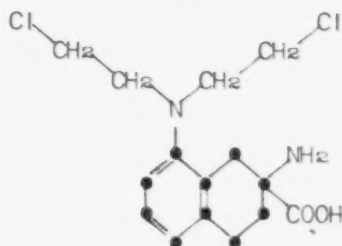
These results show that there is little predictability, at least for these two systems, in the animal tumor screens as a random sampling would give a PV+ value of 0.50.

In a cooperative study with Cancer Therapy Evaluation Program, we are currently establishing an information data base management system designed to provide administrative and scientific information in the form of reports concerning protocols, both active and completed, treatment modalities, diseases, dose schedules, results, investigator teams, drug inventory and use rate, etc. This information system incorporates the BRIGHT system (DEC-10) as well as the Statistical Analysis System (SAS) and WYLBUR systems on the IBM 370 facility at the Division of Computer Research and Technology.

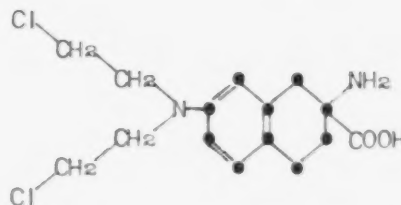
SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07104-06 LMCB																				
PERIOD COVERED October 1, 1980 to September 30, 1981																						
TITLE OF PROJECT (80 characters or less) Carrier Mediated Transport of Melphalan in Differential Cytotoxicity and Therapy																						
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SUMMARY OF WORK (200 words or less - underline keywords) The bis(chloroethyl)amine derivative of the aromatic amino acid, 2-naphthalene carboxylic acid, 2-amino was synthesized and evaluated as a new potentially less myelosuppressive <u>alkylating agent</u> . The <u>cytotoxicity</u> of 2-naphthalene carboxylic acid, <u>2-amino-7-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro</u> toward murine L1210 leukemia cells was equal to that of <u>L-phenylalanine mustard (L-PAM)</u> , however, no improvement in the therapeutic index was observed.																						

Objective:

This project is designed to evaluate the antitumor specificity of two bicyclic α -amino acid nitrogen mustards (see structures below) designed to be transported by an amino acid transport system (System L) which is lacking in cells isolated from a host-sensitive tissue, the bone marrow progenitor cells of the white cell series.



2-Naphthalene carboxylic acid,
2-amino-8-[bis(2-chloroethyl)-
amino]-1,2,3,4-tetrahydro



2-Naphthalene carboxylic acid,
2-amino-7-[bis(2-chloroethyl)-
amino]-1,2,3,4-tetrahydro

Methods Employed:

A. Murine L1210 leukemia cells are exposed to the respective cytotoxic agent and potential competitors in the appropriate medium for 30-40 minutes. Cytotoxicity is determined by clonal growth of surviving cells in soft nutrient agar.

B. Cellular Transport Studies. Logarithmic phase murine L1210 leukemia cells are removed from growth medium and incubated in Dulbecco's phosphate buffered saline containing bovine serum albumin and glucose with the respective radio-labeled substrate and potential competitor. After appropriate times, aliquots of the cell suspension are layered over Versilube F-50 silicone oil and centrifuged at 12,000 x g for one minute in an Eppendorf microcentrifuge to pellet the cells. Cell pellets are solubilized in sodium hydroxide and radioassayed by liquid scintillation spectrometry.

Major Findings:

A. Structural Requirements for Transport of Amino Naphthalene Carboxylic Acid by System L. Melphalan (L-PAM) is transported into murine L1210 leukemia cells by two amino acid transport systems, one of which is the classical System L which is responsible for the uptake of cyclic, branched chained and aromatic amino acids. The observation that System L is present in murine L1210 leukemia cells but absent in bone marrow progenitor cells afforded the opportunity to compare the aromatic bicyclic amino acids 1-naphthalene carboxylic acid, 1-amino and 2-naphthalene carboxylic acid, 2-amino with the aliphatic bicyclic amino acid, 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid as substrate for System L. Using [^{14}C]-labeled 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid

as a substrate specific for System L, inhibition constants for 1-naphthalene carboxylic acid, 1-amino (>10 mM) and 2-naphthalene carboxylic acid, 2-amino (5 μ M) indicated that a structural requirement existed for these amino acids to be transported by System L and suggested that the synthesis of 2-naphthalene carboxylic acid, 2-amino-(7)-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro might result in an antitumor agent which exhibits more antitumor specificity and less myelosuppression.

B. Comparative Cytotoxicity of L-Phenylalanine Mustard and 2-Naphthalene Carboxylic Acid, 2-Amino-7-[bis(2-chloroethyl)amino]-1,2,3,4-tetrahydro. The two aromatic nitrogen mustards were equally toxic towards murine L1210 leukemia cells and murine bone marrow progenitor cells and thus no improvement in therapeutic index was observed. The 8-bis(2-chloroethyl)amino derivative was found to be approximately 2-fold less cytotoxic than L-PAM in both cell systems.

Proposed Course:

The antitumor efficacy of both compounds is currently being evaluated in vivo and compared to L-PAM.

Publications:

Vistica, D.T. and Schuette, B.P.: Carrier mechanism and specificity accounting for the increase of intracellular melphalan by the basic amino acids. Mol. Pharmacol. 19: 92-96, 1981.

Vistica, D.T. Cytotoxicity as an indicator for transport mechanism: Evidence that murine bone marrow progenitor cells lack a high-affinity leucine carrier which transports melphalan in murine L1210 leukemia cells. Blood 56: 427-429, 1981.

Vistica, D.T., VonHoff, D.D. and Torain, B.: Uptake of melphalan by human ovarian carcinoma cells and its relationship to the amino acid content of ascitic fluid. Cancer Treat. Rep. (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07114-03 LMCB												
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TITLE OF PROJECT (80 characters or less) Carrier Mediated Transport of Nucleosides in Differential Cytotoxicity and Therapy														
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TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER:												
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SUMMARY OF WORK (200 words or less - underline keywords) The toxicity of <u>showdomycin</u> toward both L1210 cells and bone marrow progenitor cells <u>in vitro</u> can be blocked by the nucleoside transport inhibitor 6-[(2-hydroxy-5-nitrobenzyl)-thio]purine-9-β-D-ribofuranoside. However, the cytotoxicity of the chemotherapeutic agent, <u>5-azacytidine</u> cannot be reduced in either cell line.														

Objectives:

Although nucleoside metabolism and nucleoside antimetabolites figure prominently in cancer chemotherapy, the possible role of nucleoside transport in differential cytotoxicity has not been investigated. Our objective is to evaluate possible differences between the nucleoside transport system of tumors and normal renewal systems such as bone marrow progenitor cells in order to increase chemotherapeutic specificity.

Methods Employed:

Cells are exposed to cytotoxic and protective agents in appropriate medium for various times. The surviving fraction of cells is then determined by clonal growth in soft nutrient-agar. For transport studies, cells are removed from growth medium and incubated in Dulbecco's phosphate buffered saline containing bovine serum albumin, glucose and isotopically labeled compound. After appropriate exposure times, the distribution of the labeled compound is determined by liquid scintillation spectrometry. Synthesis of appropriate analogs is performed and purity of products evaluated by thin layer chromatography.

Major Findings:Transport Carrier Dependence and Independence Among Cytotoxic Nucleosides

The nucleoside analogs showdomycin and 5-azacytidine are cytotoxic toward L1210 tumor cells and bone marrow progenitor cells. The toxicity of showdomycin can be blocked by the nucleoside transport inhibitor 6-[(2-hydroxy-5-nitrobenzyl)-thio]purine-9- β -D-ribofuranoside, which interferes with its uptake. In contrast, the toxicity of 5-azacytidine toward both L1210 cells and bone marrow progenitor cells was not even reduced by the transport inhibitor. Intracellular cytotoxic concentrations of 5-azacytidine may be achieved by limited diffusion and phosphorylation, reducing the requirement for the nucleoside transport carrier.

Proposed Course:

It is suggested that a purine nucleoside carrying a nitrogen mustard group at the 6-position and having the 5'-position methylated to prevent phosphorylation may be an interesting candidate for selective toxicity brought about by differential competition for transport.

Publications:

Uehara, Y., Fisher, J.M. and Rabinovitz, M.: Showdomycin and its reactive moiety, maleimide: A comparison in selective toxicity and mechanism of action in vitro. Biochem. Pharmacol. 29: 2199-2204, 1980.

Uehara, Y. and Rabinovitz, M.: Transport dependent membrane damage and the irreversible inactivation of nucleoside transport by showdomycin. Biochem. Pharmacol. (in press), 1981.

Numao, N., Hemmi, H., Naujokaitis, S.A., Rabinovitz, M. and Beisler, J.A.: Showdomycin analogs: Synthesis and antitumor evaluation. J. Med. Chem. (in press), 1981.

Ayukawa, S., Fisher, J.M. and Rabinovitz, M. Proteolytic susceptibility of hemoglobin synthesized in the presence of amino acid analogs. Mol. Pharmacol. (in press), 1981.

Naujokaitis, S.A.: Thiamine protection of murine L1210 leukemia cells against mechlorethamine cytotoxicity and its relation to the choline uptake system. Res. Commun. Chem. Pathol. Pharmacol. (in press), 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07117-02 LMCB																
PERIOD COVERED October 1, 1980 to September 30, 1981																		
TITLE OF PROJECT (80 characters or less) Melphalan Resistance by the L1210 Leukemia and Approaches Toward Its Reduction																		
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SUMMARY OF WORK (200 words or less - underline keywords) Melphalan resistant L1210 cells were also resistant to the cytotoxic action of the poorly permeable mercurial, <u>p-chloromercuribenzenesulfonate</u>. This cytotoxic resistance was reflected in <u>resistance to membrane damage</u> by the mercurial indicated by increased Na^+ and Ca^{++} permeability and to cell swelling and lysis. The implication of an <u>exofacial sulfhydryl group</u> in these interactions is apparent, and it is suggested that this group is either less active or less accessible in the resistant cell.																		

Objectives:

The development of resistance to alkylating agents during cancer chemotherapy is a principal factor in their limitation as therapeutic agents. We have initiated a multidisciplinary approach to explore the mechanism of resistance to a clinically useful alkylating agent, melphalan, in an attempt to determine the biochemical mechanism of resistance and develop regimens to overcome or reduce it.

Methods Employed:

Growth parameters of primary cultures of *in vivo* lines of melphalan sensitive and resistant L1210 leukemia cells have been established. Radiochemical analytical techniques, including thin layer chromatography and gel filtration for identification of possible melphalan metabolites have been developed and histochemical and enzymatic analytical techniques for determination of cellular components and metabolic pathways have been adapted to our requirements. Uptake studies using isotopes of mercury, sodium, potassium and calcium are performed. The Coulter Channelyzer has been particularly useful in monitoring cell volume changes.

Major Findings:

The availability of the melphalan sensitive and resistant lines of L1210 cells in primary culture permitted an analysis of their sensitivity to a variety of agents which could serve as biochemical probes. Growth of cell types were equally inhibited by a wide variety of cytotoxic agents including microtubule and microfilament poisons. Although last year's report indicates that a sulfhydryl basis for resistance is apparent, both cell types were equally sensitive to the sulfhydryl reagents iodoacetamide and N-ethylmaleimide. However, the melphalan resistant line was also 3 to 5 times resistant to the poorly permeable mercurial p-chloromercuribenzenesulfonate (PCMBs). This differential cytotoxicity was also established by clonal growth and is accompanied by a major swelling and lysis of the cells, which could be closely monitored with a Coulter Channelyzer.

The "impermeant maleimide" in which glutathione is coupled to bismalimidomethyl ether [Abbott, R.E. and Schacter, D., J. Biol Chem. 251, 7176 (1976)] completely blocked PCMBs-induced swelling and lysis of the tumor cells. These observations confirm our view that melphalan resistance is associated with the reduced availability of an exofacial membrane sulphydryl group. Reaction of this group with the mercurial induces a cascade of further reactions with internal sulfhydryl groups, which could be followed by the increasing rate of reactivity of sensitive cells with Hg^{203} -labeled PCMBs.

The initial binding of ^{203}Hg PCMBs was the same for melphalan sensitive and resistant cells, and no increase in release of label was seen in the resistant cells upon incubation in mercury-free medium. Treatment of cells with $5\text{ }\mu\text{M}$ PCMBs resulted in 4 times as much uptake of $^{22}\text{Na}^+$ by the sensitive cells. At $50\text{ }\mu\text{M}$ PCMBs, three times as much $^{45}\text{Ca}^{++}$ was taken up by the sensitive cells. However, $^{42}\text{K}^+$ release was not an indicator of PCMBs differential activity in

the melphalan sensitive and resistant lines. When both cell lines were exposed to mechlorethamine at 25 μ M for 30 min, five times as much $^{45}\text{Ca}^{++}$ was taken up by the sensitive L1210 cells. These differential responses to PCMBs and alkylating agents as measured by survival and membrane permeability to ions may be related to the biochemical basis of resistance.

Proposed Course:

The use of appropriate sulfhydryl reactive agents in combination treatment with melphalan will be investigated.

Drug Interactions Section

Laboratory of Medicinal Chemistry and Biology

October 1, 1980 - September 30, 1981

Objectives

The interaction between a drug and tissue receptors which ultimately produces a pharmacologic or toxicologic response is the product of a large number of factors. For example, the distribution of a drug within the body depends upon relative organ perfusion, affinity of the drug for binding sites within different organs, extent of binding of the drug to plasma proteins, and the rate of drug excretion into the urine, bile, and exhaled air. Moreover, the metabolic clearance of many drugs from the body depends upon their relative rates of biotransformation to products which are more polar and more rapidly excreted. Thus, absorption, tissue distribution, binding, metabolism and excretion determine the steady-state plasma drug concentration under specified conditions and thus determine the pharmacologic or toxicologic response. Since alterations in these factors are known to either diminish or intensify biologic responses, the need to quantitatively assess these parameters is of obvious therapeutic significance. Studies have been continued in the Drug Interactions Section to investigate these parameters in a variety of animal species with respect to xenobiotics in general, and oncolytic agents in particular. A large body of evidence has linked drug metabolism to drug disposition and the duration of drug action. Only recently have data accumulated which emphasize the important role of drug metabolism in drug-induced toxicity. Recent findings have demonstrated that many relatively inactive foreign chemical compounds can be enzymatically activated in vivo to what might be termed ultimate toxicants.

Because of the diversity of interests of members of the Section, a large variety of research projects continue to be conducted. For organizational purposes, these projects may be grouped under three general headings which are described in detail in subsequent pages of this report.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CM 07119-02 LMCB																																								
PERIOD COVERED October 1, 1980 to September 30, 1981																																										
TITLE OF PROJECT (80 characters or less) Interactions of Antineoplastic Agents in Biological and Chemical Systems																																										
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SUMMARY OF WORK (200 words or less - underline keywords) In recent years, it has become common practice, particularly in clinical oncology, to simultaneously administer several drugs to patients for therapeutic purposes. A vast literature, both experimental and clinical, has shown that the presence of one drug may markedly influence the intensity, duration, and pharmacologic effects of another drug. Such <u>drug interactions</u> may have a variety of causes: they may result from alterations in <u>drug absorption</u>, in the <u>binding</u> of drugs, in tissue or to <u>plasma proteins</u>, which may directly or indirectly influence <u>drug distribution</u>, <u>drug metabolism</u>, or <u>drug clearance</u> via the bile or urine. Our major efforts are directed at understanding how drugs interact with each other and with biological organisms so that human drug therapy can be based on more rational grounds.																																										

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. Interaction Between Cis-Platinum and Possible Tissue Receptors. The mechanism of the binding of cis-Pt to nucleic acids is reasonably well established but the mechanism by which organ-specific toxic effects are produced is less well understood. In an attempt to more fully understand these mechanisms we have investigated several potential receptor molecules that may be involved in cis-Pt-induced toxicity.

Because of the highly electrophilic nature of cis-Pt, sulfhydryl-containing molecules have been implicated as binding sites for cis-Pt. We investigated the in vivo and in vitro effects of cis-Pt on glutathione and glutathione-dependent enzyme activities. The only effect observed in liver was a mild decrease in glutathione levels. In kidney, however, a time-dependent increase in glutathione was observed, with levels five times normal being achieved 12 days after treatment. A concomitant decrease in glutathione reductase was also observed. Effects on glutathione S-transferases were substrate specific with S-aryltransferase increased and epoxide transferase decreased. No change in transferase activity was observed when cis-Pt was added in vitro to incubation mixtures.

In vitro effects of cis-Pt on ATPases have been the basis for proposing ATPase as the sensitive receptor for platinum in kidney. We treated rats with cis-Pt and then studied the activity of various ATPases in mitochondria, microsomes, and plasma membranes of liver and kidney. No alterations in any ATPase were observed in either tissue at a near-lethal dose of cis-Pt. Effects of cis-Pt on ATPase activity in duodenum and ileum of the rat small intestine were also investigated.

Platinum levels in the microsomal fractions of tissues from cis-Pt treated rats have been shown to be very high. We therefore investigated the effect of treatment with cis-Pt on hepatic and renal mixed function oxidase activity. Drug metabolizing enzyme activities in both tissues were found to be routinely increased by cis-Pt treatment but with no concomitant increase in protein content or cytochrome P-450 levels. Lipid peroxidation was increased 7-20 times in liver throughout the 12 day course of the study but no change in this parameter was observed in kidney.

B. Further Studies on CCNU (1-(2-chloroethyl)-3-(cyclohexyl)-1-nitrosourea): Its Effects on Heme Metabolism and Lipid Peroxidation in Relation to Its Inhibition of Hepatic Microsomal Mixed-Function Oxidation. A single intraperitoneal dose of CCNU, to male rats, has been shown to cause a generalized inhibition in hepatic mixed-function oxidase parameters during the first week after treatment, followed by a prolonged depression of cytochrome P-450 and metabolism of Type I substrates, such as biphenyl and benzphetamine. Since many chemicals that degrade cytochrome P-450 are also known to cause alterations in the pattern of heme metabolism, the effects of CCNU on various heme biosynthetic and degradative enzymes were investigated to relate such changes to the observed decrease

in hepatic cytochrome P-450 content. In a time course study, the changes in heme enzymes also proceeded in a biphasic fashion as observed with the mixed-function oxidases. There was an initial increase in the biosynthetic enzymes, δ -ALA synthetase and δ -ALA dehydratase, and the degradative enzyme, heme oxygenase. The extent of stimulation of these enzymes, however, fell sharply within 5 days post-treatment but δ -ALA synthetase was restimulated and heme oxygenase remained significantly above control values up to the end of the ten-week study. The initial stimulation of these heme enzymes appears to precede changes in the levels of cytochrome P-450 and may, therefore, be partly responsible for the latter observation. However, other factors such as alterations in protein metabolism or affinity of apo-cytochrome P-450 for heme may also be involved.

Lipid peroxidation is believed to be involved in toxicity caused by drugs such as adriamycin and paraquat. In the case of carbon tetrachloride, destruction of cytochrome P-450 may be linked to stimulation of lipid peroxidation. Since CCNU, after its administration to rats, stimulates lipid peroxidation, the decrease in cytochrome P-450 may result from such a process. Therefore, protection against lipid peroxidation, e.g. by vitamin E administration, should prevent destruction of the hemoprotein. However, our study showed that although vitamin E can effectively block the lipid peroxidation caused by CCNU, mixed-function oxidases were still depressed and did not differ from animals treated with CCNU alone. Concomitantly, vitamin E treatment did not appear to decrease hepatotoxicity, by measurements of SGPT and SGOT levels, in CCNU-treated animals. These results show that the various changes in hepatic mixed-function oxidase system and hepatotoxicity caused by CCNU are not initiated by lipid peroxidation.

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Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

A. Bioavailability of Intramuscular Δ^9 -Tetrahydrocannabinol (Δ^9 -THC). Δ^9 -THC is an antiemetic used in patients undergoing chemotherapy. The usual route of administration for this purpose is oral, which has obvious disadvantages in severely nauseated patients. We have studied the bioavailability, distribution and excretion of Δ^9 -THC following IM or IV administration to rabbits. IM administration produced an Area Under the Curve (cxt) of approximately 1/3 of the iv value. Decay of plasma radioactivity after iv administration was characterized by three distinct phases with half times of 36, 538, and 1287 minutes. Long peak plasma levels (15-30 hours) were common after im administration. Elimination rate and percent excreted were similar for both routes, with 40-50% of the administered dose excreted in urine and 10-20% excreted in feces during 72 hours. In all but one rabbit treated im, less than 7% of the administered dose was recovered in the muscle 48 or 72 hours after dosing. Distribution of radioactivity 24, 48, and 72 hours after administration was qualitatively similar with both routes of administration. Rabbits treated im demonstrated considerably less CNS stimulation than did the rabbits treated iv.

B. Damage to Clara Cells and Inhibition of Pulmonary Mixed-Function Oxidation by Naphthalene. The administration of naphthalene has been shown previously to elicit selective damage and necrosis of the Clara cells of mouse lung. Since these cells are believed to be associated with the mixed-function oxidase system, their destruction by naphthalene would be expected to impair the activities of these enzymes, and the extent of such changes should be dependent on the severity of cellular damage. Our data are consistent with such reasoning in that naphthalene produced selective inhibition in pulmonary monooxygenases 24 hours after treatment and histological studies revealed dose-dependent damage to Clara cells in the mouse. Naphthalene did not produce an effect on hepatic monooxygenases and no remarkable changes were observed in livers of the same animals. Thus, the view that cytochrome P-450 catalyzed activities occur in Clara cells are compatible with the results obtained in our experiments.

Currently, a more thorough study to investigate the duration of the pulmonary effects caused by naphthalene (and also its recovery), and changes both at the light and electron-microscopic levels in lung and liver are being examined.

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TITLE OF PROJECT (80 characters or less) Investigations on the Involvement of Reactive Forms of Oxygen in Drug-Induced Pulmonary Toxicity																		
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SUMMARY OF WORK (200 words or less - underline keywords) The drug-induced generation of <u>reactive forms of oxygen</u> (superoxide anion, <u>hydroxyl radical</u> and <u>singlet oxygen</u>) can contribute to drug <u>cytotoxicity</u> through attack of reactive oxygen species on <u>intracellular targets</u> (nucleic acids, lipids, proteins) and/or through reactive oxygen-mediated activation of the drug to a <u>reactive intermediate</u>. There are an increasing number of reports describing the <u>pulmonary toxicity</u> of antineoplastic agents. Reactive forms of oxygen have been implicated in the action of several of these agents. The present projects were designed to evaluate this hypothesis in order to better understand the possible biochemical and molecular mechanisms which contribute to this pulmonary toxicity.																		

Methods Employed:

Standard enzymatic and analytical techniques have been utilized.

Major Findings:

Life-threatening pulmonary toxicity as a result of anticancer drug therapy is becoming increasingly recognized. It is also becoming apparent that because of the inherent molecular properties of some antineoplastic agents, reactive oxygen may be involved in the cytotoxic reaction(s) to lung cells. Two possible reactions are microsomal lipid peroxidation and DNA damage.

A. In Vitro Stimulation by Paraquat of Reactive Oxygen-Mediated Lipid Peroxidation in Rat Lung Microsomes. In this study, the herbicide paraquat was used as a prototype of redox cycling chemicals which elicit pulmonary toxicity; we wished to assess in vitro conditions necessary to stimulate lipid peroxidation in rat lung microsomes. This reaction has been studied by previous investigators and has resulted in conflicting observations. However, it was our opinion that these discrepancies arose because of methodological inconsistencies, i.e., failure to provide sufficient reducing equivalents (NADPH); too short of an incubation period; and limiting atmospheric conditions. To this end, we have utilized an in vitro system which takes into account these factors and have observed that, indeed, paraquat can significantly stimulate lipid peroxidation in microsomes isolated from either rat or mouse lungs. Moreover, this reaction is dependent on reactive oxygen as indicated by the significant inhibition by superoxide dismutase. Thus, we have developed an in vitro system which not only demonstrates paraquat is capable of stimulating lipid peroxidation in lung microsomes but is applicable to the study of the interaction of lung-toxic antineoplastic agents with pulmonary microsomes.

B. Studies on the In Vitro Interaction of Mitomycin C, Nitrofurantoin and Paraquat with Pulmonary Microsomes: Stimulation of Reactive Oxygen-Dependent Lipid Peroxidation. Mitomycin C, like nitrofurantoin and paraquat, is capable of being reduced by microsomes, in the presence of NADPH, to a reduced radical species. However, under aerobic conditions these reduced radical species rapidly reoxidize with the formation of the parent compound and superoxide. Since there has been an increased number of clinical reports that mitomycin elicits pulmonary toxicity, in vitro experiments were performed to evaluate the capacity of mitomycin C (MC), relative to nitrofurantoin and paraquat (PQ), to stimulate pulmonary microsomal lipid peroxidation. It was observed that the interaction of MC, NF or PQ with rat or mouse lung microsomes in the presence of an NADPH-generating system and an O₂ atmosphere resulted in significant lipid peroxidation. All three compounds demonstrated similar concentration-dependency, similar time courses and the ability to generate lipid peroxidation-associated chemiluminescence. The stimulation of lipid peroxidation by MC, NF or PQ was significantly inhibited by superoxide dismutase, glutathione, ascorbic acid and EDTA, agents which either scavenge reactive oxygen and/or prevent the generation of secondary reactive oxygen metabolites. In addition, the ability of MC or NF, but not PQ, to stimulate lipid peroxidation was significantly reduced following preincubation with microsomes and NADPH under N₂ (15-20 min) prior to incubation under O₂. During this period under N₂, MC and

NF underwent reductive metabolism of their quinone and nitro-moieties, respectively. Thus it appears under aerobic conditions, the pulmonary microsomal-mediated redox cycling of MC, NF and PQ is accompanied by the stimulation of reactive oxygen-dependent lipid peroxidation.

C. In Vitro Studies on the Interaction of Bleomycin A₂ with Pulmonary Microsomes: Characterization of Factors which Affect Bleomycin-Mediated DNA Damage. The administration of bleomycin is accompanied by dose-limiting pulmonary toxicity. Like many antineoplastic agents DNA is believed to be the cellular target of bleomycin's action. It has been proposed also that the interaction of bleomycin with Fe²⁺ forms a potent redox cycling system. We have studied the interaction of bleomycin A₂ with pulmonary microsomes to determine if it results in lipid peroxidation and to determine if this interaction results in deoxyribose cleavage. Unlike mitomycin C, the incubation of bleomycin with rat lung microsomes, in the presence of NADPH and O₂ does not result in lipid peroxidation. However, if DNA is present significant cleavage of the deoxyribose moiety results. This bleomycin-mediated DNA cleavage is NADPH-dependent (although NADH can also serve as an electron donor), oxygen-dependent and dependent on the type of nucleic acid employed. Furthermore, it was observed that incubation of bleomycin with microsomes in the absence of DNA resulted in the inactivation of bleomycin upon subsequent addition of DNA. This inactivation of bleomycin by lung microsomes is also oxygen and NADPH dependent. Preliminary studies have demonstrated that purified NADPH cytochrome P-450 reductase can also mediate bleomycin-DNA chain breakage.

D. Studies on the In Vitro Interaction of Bleomycin A₂ with Pulmonary Microsomes: Facilitation of Bleomycin-Mediated DNA Cleavage by Reactive Oxygen. It has been proposed that reactive oxygen is involved in the cleavage of DNA by bleomycin. We have evaluated whether reactive oxygen contributes to bleomycin-mediated DNA cleavage following interaction with rat lung microsomes by two approaches: (1) by examining the effects of reactive oxygen scavengers and (2) by examining the effects of redox cycling chemicals on this reaction. It was observed that superoxide dismutase and dimethylurea, a hydroxyl radical scavenger, significantly inhibited bleomycin-mediated DNA cleavage implicating the involvement of reactive oxygen. This reaction was also inhibited by cytochrome c, NBT, EDTA and glutathione. On the other hand, ascorbic acid significantly enhanced this reaction. The addition of the redox cycling chemicals mitomycin C, nitrofurantoin or paraquat also significantly enhanced this reaction. The enhancing effect of these redox cycling chemicals was significantly inhibited by superoxide dismutase. Furthermore, bleomycin significantly inhibited the stimulation of lipid peroxidation by these chemicals implying that reactive oxygen was diverted to the bleomycin-DNA complex rather than to microsomal phospholipids. This study confirms that reactive oxygen is involved in bleomycin-mediated DNA cleavage by a biological system.

E. Similarities in the Interaction of Bleomycin A₂ with Fe²⁺ to the Interaction with Lung Microsomes: Development of a Model that a Reactive Bleomycin Intermediate is Involved in Bleomycin-Mediated DNA Cleavage. The interaction of DNA and bleomycin with Fe²⁺ results in deoxyribose cleavage. In the absence of DNA however this interaction results in the inactivation of bleomycin and is also accompanied by the generation of chemiluminescence (CL). Interestingly, the time courses of these three reactions are similar implying that a common mechanism may be involved. We have hypothesized that the CL is indicative, at least in part, of an electronically excited labile state of bleomycin.

We have recently observed that the interaction of bleomycin with lung microsomes also results in chemiluminescence. The time course of this CL is similar to the time course for both bleomycin-mediated DNA cleavage and bleomycin inactivation following the interaction of bleomycin with microsomes. Preliminary studies have indicated that the addition of paraquat to the microsomal system not only enhances bleomycin-mediated DNA cleavage but also stimulates bleomycin-inactivation and bleomycin-chemiluminescence. From these studies we propose that following the interaction of bleomycin with either chemical or biological free radical generating systems some portion of the bleomycin molecule is activated to an electronically excited state which can pass this excess energy to DNA, resulting in deoxyribose cleavage, or in the absence of DNA decomposes to an inactivated state. Studies are currently in progress to develop this model as to better understand the molecular mechanisms involved in bleomycin's chemotherapeutic reaction and also cytotoxic reactions to lung cells.

Significance to Biomedical Research and the Program of the Institute:

As stated under objectives, drug interactions are known to profoundly influence the disposition of drugs and thus may determine whether a normal therapeutic dose of drug will become either a sub-threshold or toxic dose. The significance of this knowledge to cancer chemotherapy, particularly in the era of combination drug administration, cannot be over emphasized.

Proposed Course:

The projects being pursued are only partially complete. In addition to examining in more detail the influence of antineoplastic agents upon the metabolism, distribution, and binding of drugs and their toxicity, we envision studying the metabolic fate of antineoplastic agents.

Publications:

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